Reproduction in the Western Mosquitofish, *Gambusia affinis affinis* (Baird & Girard), and Its Use in Mosquito Control

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Indiana University

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY OF MICHIGAN

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REPRODUCTION IN THE WESTERN MOSQUITOFISH, *GAMBUSIA AFFINIS AFFINIS* (BAIRD & GIRARD), AND ITS USE IN MOSQUITO CONTROL

INTRODUCTION

This study of the natural history of the mosquitofish, Gambusia, outside its indigenous area embraces several somewhat diverse yet intimately related biological aspects. The productivity of this poeciliid in ponds, including the factors of age, growth, maturity, reproduction, and survival, is given particular emphasis. The actual productivity in various waters is analyzed with primary emphasis on one pond in central Illinois where conditions for reproduction, survival and growth exceeded all expectations and undoubtedly approached the optimum.

Another phase of the study treats the establishment and acclimatization of Gambusia into northern Illinois and thence into Michigan.

The data on productivity and on the northward spread are brought to bear on the use of this fish as a mosquito-controlling agent in the northern states. Some data are given on the efficacy of Gambusia in the control of these noxious and dangerous insects in Michigan.

The aspects of natural history of Gambusia that are given detailed consideration include:

1. The size range and rate of growth of individuals of each sex.
2. The size and age of males and females at first maturity.
3. The length of the reproductive period in male and female mosquitofish.
4. The number of broods to which females give birth during one season.
5. The number of individuals in each of the broods.
6. The length of the period of gestation.
7. The approximate life span of the species in nature.
8. The sex ratio at birth and variations in that ratio during the year particularly as related to age and size.
9. The establishment of the mosquitofish in northern Illinois and thence into Michigan.
10. The conditions required for a population to maintain itself in a climate more severe than that of its natural range.
11. The effectiveness of the mosquitofish as an agent for the control of mosquitoes.

Preliminary collections of Gambusia were made from 5 small woods ponds in the Forest Preserve District of Cook County, Illinois, during August 1938. The fish had been placed in those bodies of water as a mosquito-control measure by the Des Plaines Valley Mosquito Abatement District. Those samples indicated that there were considerable local differences in the various aspects of the life history of this poeciliid and that further collections were desirable. Accordingly, subsequent collections of mosquitofish were made in an attempt to determine the life span of the species in nature and to secure information on its life history and ecology under natural conditions outside its indigenous territory.

During the summer of 1939 mosquitofish were collected at approximately monthly intervals in an attempt to determine the number of broods born during a season, the number of individuals per brood, and any changes that might have occurred in the sex ratio at different times during the summer. The specimens were taken from two ponds, the Argonne Woods Pond and the Sanitary District Lake, in the Chicago region. Both of these ponds had maintained populations of Gambusia for at least 3 years and neither had been stocked with additional fish in 1939.

In May 1940, a pond near Mt. Zion, Macon County, Illinois, was stocked with virgin female and male Gambusia in the hope that collections later in the summer would yield information concerning the length of the period of gestation, the time necessary for the newborn to reach maturity, the sex ratio in a newly established population, and the total production of fish in the pond. Studies on the length of the gestation period and sex ratio at birth were also carried on in the laboratory.

Mosquitofish were introduced into several waters in southeastern Michigan in June 1941, in an effort to establish the species in that area. Observations were made on the life history, ecology, and the conditions required for a population of Gambusia to maintain itself in a climate more severe than that of its natural range. Small samples of fish were taken from time to time for use in the laboratory, but no large collections, similar to those made in Illinois, were taken because of the possibility of seriously depleting the populations as they became established. In addition, many mosquitofish were introduced into natural waters in different parts of the lower peninsula of Michigan as a mosquito-control measure, and this in itself made the killing of large numbers of fish undesirable.

During the summers of 1943 and 1944, experiments on the effectiveness of the mosquitofish as an agent for the control of anopheline mosquitoes were carried on in experimental ponds of the State Fish Hatchery at Drayton Plains, Michigon.

ACKNOWLEDGMENTS

This is a revision of a dissertation accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Michigan in 1945.

My gratitude is due the Illinois Natural History Survey, the Forest Preserve District of Cook County, Illinois, the Des Plaines Valley Mosquito Abatement District, and the Institute for Fisheries Research of the Michigan Department of Conservation for the use of many facilities and to various members of the staffs of these organizations for suggestions and advice and for assistance in collecting material. I owe my thanks to Dr. David H. Thompson, of the Forest Preserve District of Cook County, Illinois; Dr. G. W. Bennett of the Illinois Natural History Survey; Professor L. A. Adams of the University of Illinois; Mr. J. Lyell Clarke of the Des Plaines Valley Mosquito Abatement District; Professor G. R. LaRue of the University of Michigan; Dr. Ralph Hile of the U. S. Fish and Wildlife Service; and many others who offered valuable criticisms and suggestions during the course of the work. The Horace H. Rackham School of Graduate Studies of the University of Michigan gave financial aid for the experiments on mosquito control. I am particularly indebted to Professor Carl L. Hubbs of the Scripps Institution of Oceanography for supervising the major portion of the work and for the many invaluable hints in assembling the data and preparing the manuscript.

MATERIAL AND METHODS

Taxonomy of Fish Studied

The kind of Gambusia studied during the present investigation is the western mosquitofish, *G. affinis affinis* (Baird & Girard). The genus belongs to the viviparous family Poeciliidae of the order Cyprinodontes. This little topminnow, so called because of its habit of seeking food at the surface is light olive with speckled fins, but is without any conspicuous color markings. The adult females range from 1 to 2½ in total length, whereas the mature males have an average total length of about 1¼ inches. As in other poeciliids, mature individuals exhibit sexual dimorphism; the anal fin of the female is small and rounded whereas that of the male is modified into an elongated intromittent organ, the gonopodium. Figure 1 shows an adult male and female *Gambusia a. affinis.*

The genus Gambusia was established by Poey (1854), who simultaneously described two Cuban species *Gambusia punctata* and *Gambusia punctic-ulata.* The generic name was derived from the provincial Cuban word Gambusino as indicated by Poey, "El nombre debe su etimología á la palabra provincial cuban *Gambusino,* que significa *nada,* con idea de chasco ó burla: así se dice pescar Gambusinos quando no se pesca nada." "The name owes its etymology to the provincial cuban word *Gambusino,* which denotes *nothing,* in the jocular or scoffing sense: so it is said to fish Gambusinos is to catch nothing." The logotype of the genus, *G. punctata,* was selected by Jordan & Gilbert (1883).

The species with which this paper is concerned was described by Baird & Girard (1854) simultaneously as *Heterandria affinis,* from the Medina and Salado rivers in Texas, and as *Heterandria patruelis,* from the hydrographic basin of the Nueces River in the same state. Evermann & Kendall (1894), who first recognized the identity of the two nominal species, *affinis* and *patruelis,* of identical date, selected the former name.

Regan (1913) listed *Gambusia holbrookii* ranging from Virginia to Alabama, *G. patruelis* from Florida to Texas, and *G. affinis* from Florida to Tampico and the lower Mississippi Valley. Gieser (1923) held that all forms of Gambusia in the United States, with the exception of the strikingly dissimilar species from Texas that he listed as *G. senilis,* are of the species *G. affinis.* He regarded *G. holbrookii* and *G. patruelis* as being merely varieties or entirely synonymous and without taxonomic standing. He mentioned, however, 3 types of *G. affinis* that he called "Eastern form" found along the Atlantic coastal plain, "Central form" from the central Mississippi Valley region, and, "Western form" of the Southwest.

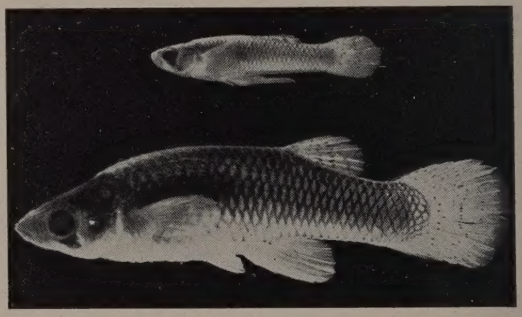

FIG. 1. Adult male (above) and female (below) *Gambusia affinis affinis.*

Hubbs (1926) divided the genus into four subgenera: (1) Heterophallina; (2) Gambusia, including, under the misidentification of *G. affinis,* the form referred to *G. senilis* by Geiser; (3) Arthrophallus, including only the species *G. patruelis,* which ranges from southern Indiana and Illinois to the Gulf of Mexico and along the Gulf Coast from Louisiana to Tampico; and (4) Schizophallus, having as the type species *G. holbrookii,* which is very similar to *G. patruelis* and occurs in the Atlantic Coast drainage from New Jersey to Florida, and in the adjacent Gulf Drainage of Florida and Georgia.

Following the introduction of the mosquitofish into Europe, zoologists encountered difficulties in the systematics of the species involved. Information received from Professor Carl L. Hubbs prompted Lindberg (1934) to state that only one species of Gambusia, viz. *G. affinis,* had been transported from the United States for use in mosquito control, and that the spe-

cies was divided into two distinct subspecies: *Gambusia affinis affinis* (Baird & Girard) inhabiting the lower Mississippi River drainage from St. Louis to Louisiana on the south, and west along the Gulf Coast to the Rio Grande, and *Gambusia affinis holbrookii* (Günther) ranging from southern New Jersey along the Atlantic Coast to and including Florida and along the Gulf Coast to Alabama. D'Ancona (1939) published a table of characters that he received from Professor Hubbs which pointed out the bases for the taxonomic separation into subspecies. The confusion regarding the application of the name *affinis* had been eliminated by the examination of type material.

The natural range of *Gambusia a. affinis* extends along the Gulf Coast from the mouth of the Rio Grande as far west as Bay St. Louis, Mississippi, and through the Mississippi River and its tributary waters as far north as central Illinois. According to Forbes & Richardson (1920), "This little top-minnow, fairly common in extreme southern Illinois, has been taken by us outside that region only from Quincy, Meredosia, and Pekin." That it is indigenous to that region is indicated by the fact that Jordan & Gilbert (1882) described *Zygonectes inurus* (synonymous with *Gambusia a. affinis*) from the Cache River, Illinois. Luce (1933) reported taking mosquitofish in five collections from the Kalkaskia River between Vandalia and Venedy Station, Illinois. Dr. David H. Thompson, formerly of the Illinois Natural History Survey, in correspondence with the writer, has given the informtaion that Gambusia was taken from the Illinois River at seven places between Pekin and the mouth of the river during the summer of 1941. In Indiana, Gerking (1945) found that the distribution of *G. a. affinis* was restricted to the extreme southwestern corner of the state. The first report from much farther north was that of O'Donnell (1935), who listed *Gambusia affinis* from the Fox River basin in northeastern Illinois. As indicated later, it has been determined that this northern occurrence resulted from an introduction. Meek & Hildebrand (1910) did not mention this genus in their "Synoptic List of Fishes Known to Occur within Fifty Miles of Chicago," and Thompson & Hunt (1930) did not collect the species from the waters of Champaign County, Illinois, where the Kalkaskia River has its origin. Hubbs & Lagler (1941) did not include the genus in their "Guide to the Fishes of the Great Lakes and Tributary Waters." From this information the parallel of 40° north latitude may be given as the approximate northern limit of the natural range of Gambusia.

Gambusia a. holbrookii ranges from southern New Jersey along the Atlantic coast to the Florida Keys and along the adjacent Gulf Coast as far as Apalachicola, Florida. Between this region and the easternmost fringe of the natural range of *G. a. affinis* these two subspecies intergrade (Hubbs & Walker, MS). *Gambusia a. speciosa* is found in clear water streams tributary to the Rio Grande in Texas, in the Rio Salado and Rio Juan systems in the Rio Grande basin of northeastern Mexico, and in the upper reaches of the Conchos and Soto la Marina systems of northeastern Mexico (information from Professor Carl L. Hubbs).

Laboratory and Field Procedures

A bright warm day when Gambusia literally swarm in the shallow waters of a pond is the most advantageous time for collecting. All collections were made with a one-eighth-inch-square-mesh "Common Sense" minnow seine, 12 feet long and 4 feet deep.

All the fish were killed and fixed in a solution of 10% formalin and were later preserved in 70% alcohol.

Measurements were made to the nearest millimeter from the tip of the snout with the mouth closed to the tip of the caudal fin (total length).

All females from all collections were inspected for young and in each gravid female those embryos which had reached the "eyed stage" and were in a similar stage of development were considered as in the same brood. Actual counts were made of the number of young in the individual broods.

The sex of each fish was determined by an examination of the anal fin. Adult males are readily distinguished by the modification of the anal fin into a copulatory organ, the gonopodium. As Turner (1941: 169) pointed out, the finer structure of the anal fin provides a criterion for the distinction of sex even in very young fish:

> "Anchylosis of the joints of the rays begins basally and proceeds apically. The process consists of a gradual narrowing of the segmentation space and finally a complete disappearance of it. At the conclusion there is no trace of a division between segments and the segments are fused end to end. . . . The first visible sex differences in the fin become apparent as a result of the process of solidification. In the male fin the process ceases after the eradication of a single intersegmental space while in the female it continues during the entire life of the animal. . . . The differences between the two sexes are easily detectable and become more pronounced as solidification progresses in the fin of the female and proceeds no further in the fin of the male."

In this study, the anal fins of all fish of a lesser total length than 35 millimeters which did not have a manifest gonopodium, were inspected with the aid of a binocular microscope. Sex ratios are indicated as the numbers of females per 100 males.

During the course of this investigation of the productivity of Gambusia, 30,093 mosquitofish (19,150 females and 10,943 males) were measured and sexed. To obtain the average numbers of young per brood carried by females of various lengths at different times of the year and from diverse localities, 2,381 gravid females which yielded 114,311 eyed embryos were examined. The fish were all collected during 1938, 1939, and 1940, from several ponds in Illinois (Table 1).

TABLE 1. Numbers of Gambusia collected during 1938, 1939, and 1940, and the numbers of broods counted, from ponds in central and northeastern Illinois.

Pond	Number of fish collected	Number of broods counted
Preliminary collections 1938	1,137	272
Argonne Woods Pond 1939	9,292	600
Sanitary District Lake 1939	12,702	777
Parr's Pond 1940	6,962	732
Total	30,093	2,381

PRODUCTIVITY

The reproductive efficiency of an animal population is of primary importance in the maintenance of that population. Thus, in order that we may understand the biology of the mosquitofish more fully, and use that fish to its greatest advantage in mosqutio control, it is necessary that we have definite information concerning the factors that contribute or detract from its productivity. This is true not only within the indigenous area of the species but also in waters into which it has been introduced.

The factors that effect the reproductive efficiency have been grouped as follows: (1) the rate of growth and the maximum age and size attained by male and female mosquitofish, the size and age at first maturity, the duration of the reproductive period, and the period of gestation; (2) the fecundity of the female a shown by the number and size of the broods born during her lifetime, and the period of senility that may follow reproduction; (3) sex ratio and differential death rate. There are then considered, (4) the data on general populations in various ponds.

AGE, GROWTH AND SIZE

Age and growth studies of a considerable number of freshwater fishes have been reported in the literature. Most of the species so considered grow to a much larger size and are considerably longer-lived than Gambusia. As a result those studies do not lend themselves well to comparison with the present investigation. Even the ordinary methods of age and growth determinations are not utilizable because of the brevity of the life span of the mosquitofish. Although annuli are formed on the scales of Gambusia, the scale method for age determination is inadequate because in northern and central Illinois this fish seldom, if ever, reaches an age of two years under natural conditions.

It is generally agreed that the maximum age and size of a given species of fish is variable and is determined by the body of water in which it lives, i.e. individuals of a species in one lake may grow to a much larger size or attain a greater age than do fish of the same species in another lake. The differences in length distribution of male and female mosquitofish from different waters in the same region are illustrated by data secured from preliminary samples collected August 18-25, 1938, from 5 permanent woods ponds located in the Palos Hills region southwest of Chicago, that ranged from 3 to 6 acres in extent and had maximum depths of 3 to 5 feet. The shorelines were somewhat marshy in all ponds and each supported a heavy growth of vegetation. The length distributions varied widely from pond to pond (Table 2). In each population, however, the females averaged longer and exhibited a greater range of length than the males. All of the fish taken in these 5 collections were young of the year with the exception of 3 individuals. Scale samples from those 3 fish, one female 53 mm. long from Pond 2, and two females, 54 and 57 mm. long respectively from Pond 5, indicated, by the presence of one annulus, that they had been born during the previous summer. None of the scales from male fish in any of the collections bore annuli. The largest female taken in any of the collections was 57 mm. long and the largest male was 34 mm. long. Both were collected from Pond 5.

In order to secure more adequate information on the rate of growth and the maximum size and age of Gambusia under natural conditions in northern Illinois, collections were made May 19, June 9, July 13, August 14, September 13, and October 19, 1939, in 2 ponds that were markedly different in type of habitat and natural fertility. The Argonne Woods Pond lies northeast of the intersection of 95th Street and 100th Avenue in Palos Township one mile south and one and a half mile east of Willow Springs Village. This permanent marsh pool, 5 to 6 acres in area, has a more or less regular saucer-shaped basin with a maximum depth of 5 feet. A levee which raised the water to its present level was built along the east shore during the winter of 1937-38. A dense oak-maple woods completely surrounds the pond and approaches the shoreline in many places. A heavy growth of aquatic vegetation, consisting primarily of *Ceratophyllum demersum*, *Myriophyllum* sp., *Potomageton* sp., and *Lemna* sp., was present and there were many cattails (*Typha* sp.) at various points along the shore. This pond was considered to be high in productivity. The Sanitary District Lake, formed by a depression in the ground at the time the Sanitary and Ship Canal was built, is about 10 acres in extent and is located about half a mile west of Lawndale Avenue between the Des Plaines River and the Sanitary and Ship Canal in the village of Summit. The surrounding terrain is largely clay and limestone and the pond was not considered to be very productive. Various pairie grasses and a few cottonwood trees were the only vegetation in the immediate vicinity of the pond. No rooted aquatic plants were present. A levee constructed at the west end of the lake 10-15 years ago raised the water to its present level. The maximum depth of the lake is about 8 feet near the levee and there is a broad expanse of shallow water at the east end.

Data on age and growth of mosquitofish in a newly

TABLE 2. Number of male and female mosquitofish in collections from each of five ponds in Cook County, Illinois, August 18-25, 1938.

Total length (millimeters)	Pond 1		Pond 2		Pond 3		Pond 4		Pond 5	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
14	..	..	..	..	..	..	..	..	..	1
15	..	..	..	1	..	..	..	..	..	..
16	..	1	..	1	..	..	..	..	..	2
17	..	..	..	7	6	..	1	..	..	..
18	1	5	..	24	7	1	..	..	..	1
19	7	9	5	26	10	2	..	..	..	..
20	4	20	6	22	8	..	..	..	1	..
21	7	26	14	64	14	3	1	..	..	..
22	2	12	16	63	12	2	2	..	..	1
23	5	15	10	52	6	4	10	1	..	1
24	1	4	10	14	8	2	5	..	..	1
25	1	5	13	38	13	1	13	..	2	..
26	2	2	7	12	20	2	20	1	1	2
27	..	3	6	10	21	2	20	1	1	2
28	..	1	10	3	5	2	9	1	..	3
29	..	1	5	6	5	..	4	5	..	5
30	..	..	..	5	1	2	11	1	2	5
31	..	..	..	5	..	1	13	3	1	2
32	..	..	..	5	..	5	2	2	..	..
33	..	..	..	8	..	4	..	..	1	2
34	..	..	..	21	..	7	..	..	1	3
35	..	..	..	28	..	8	..	1	..	..
36	..	..	..	21	..	4	..	..	..	3
37	..	..	..	7	..	3	..	1	..	1
38	..	..	..	2	..	1	..	7	..	5
39	..	..	..	2	..	1	..	..	..	1
40	..	..	..	7	..	1	..	1	..	10
41	..	..	..	10	..	1	..	..	..	6
42	..	..	..	7	..	..	..	2	..	4
43	..	..	..	1	..	..	..	..	..	3
44	..	..	..	..	..	1	..	..	..	4
45	..	..	..	..	..	2	..	..	..	..
46	..	..	..	..	..	..	..	1	..	3
47	..	..	..	..	..	..	..	..	..	3
48	..	..	..	..	..	..	..	..	..	2
49	..	..	..	..	..	..	..	..	..	..
50	..	..	..	..	..	..	..	..	..	..
51	..	..	..	..	..	..	..	..	..	..
52	..	..	..	..	..	..	..	..	..	..
53	..	..	..	1	..	..	..	..	..	..
54	..	..	..	..	..	..	..	..	..	1
55	..	..	..	..	..	..	..	..	..	..
56	..	..	..	..	..	..	..	..	..	..
57	..	..	..	..	..	..	..	..	..	1
Total	30	104	102	473	136	62	111	29	12	78
Average length	21.3	21.6	23.8	25.8	23.5	31.3	26.9	33.4	27.8	36.2
Percentage of collection	22.4	77.6	17.8	82.2	68.7	31.3	79.3	20.7	13.3	86.7

established population were secured from collections from Parr's Pond during 1940. This pond, located near Mt. Zion, Macon County, was formed by constructing a dam across a shallow ravine in a pasture to provide a watering place for livestock. At the time it was stocked with fish in May, 1940, the pond was roughly triangular in shape, 75 feet long and 35 feet wide, with a maximum depth of 2 feet. During the following summer the water level fluctuated according to the amount of rainfall, and by October of the same year the size of the pond had shrunk to 50 feet in length and 20 feet in width with a maximum depth of 12 inches. There was no submerged aquatic vegetation. Probably due to the continuous

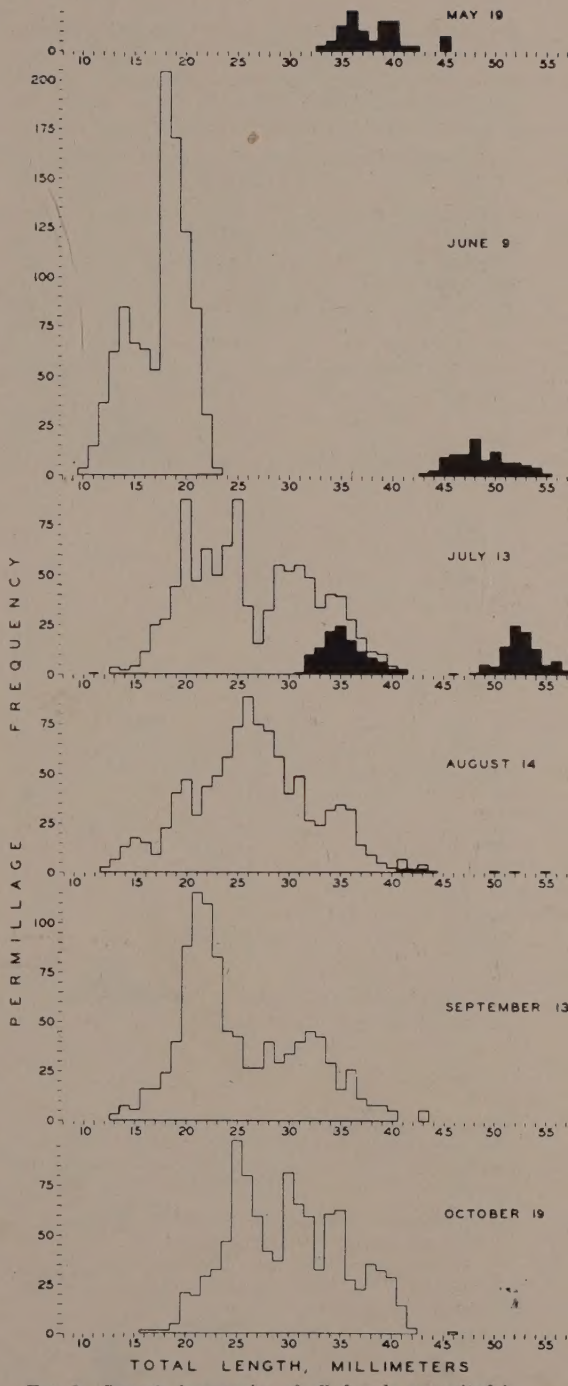

FIG. 2. Length frequencies of all female mosquitofish taken in each of six collections from the Argonne Woods Pond, Cook County, Illinois, during 1939. The shaded areas indicate the gravid females, the unshaded the non-gravid.

deposition of excreta by the cattle and hogs pastured in the area, plankton flourished throughout the summer. The surrounding terrain was gently sloping grassland with a high clay bank on the north side and a few small willows grew along the shore.

LENGTH DISTRIBUTION AND RATE OF GROWTH OF FEMALES

Respective totals of 6,231 and 8,573 female mosquitofish were taken in 6 collections each from the Argonne Woods Pond and the Sanitary District Lake and 3,600 females were secured in 4 collections from Parr's Pond. Those fish that can safely be assumed to have been present in the ponds prior to the liberation of the first brood of offspring will be referred to as the "parent" stocks and any fish born subsequent to that date will be considered as "offspring." Parent fish were present in the first 4 but were not taken in the last 2 collections from the Argonne Woods Pond, whereas such fish were present in all 6 of the collections from the Sanitary District Lake. In Parr's Pond only one fish of the original planting was found in the first collection and none were present in the subsequent collections. Offspring were taken in the last 5 collections from the Argonne Woods

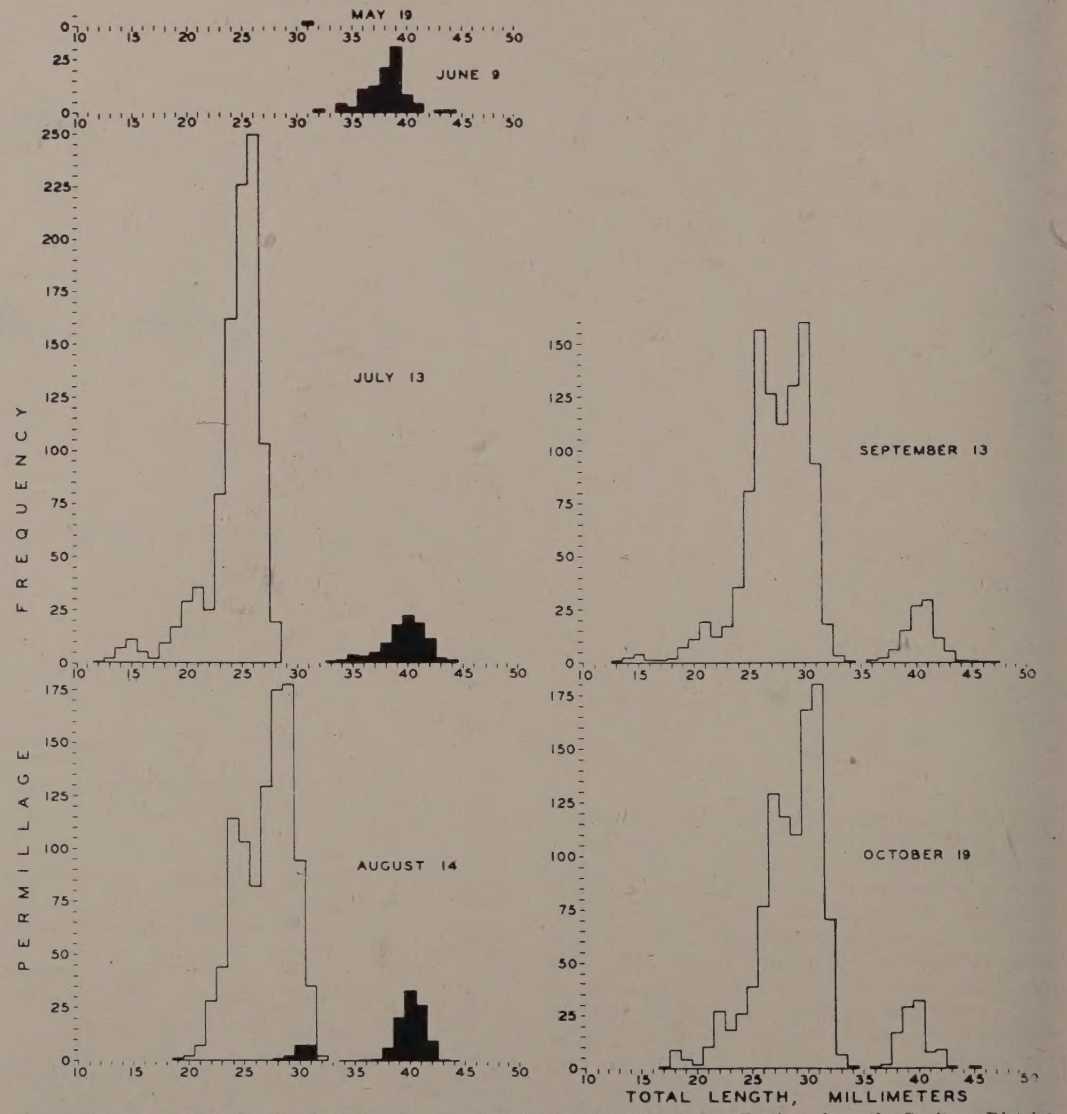

FIG. 3. Length frequencies of all female mosquitofish taken in each of six collections from the Sanitary District Lake, Cook County, Illinois, during 1939. The shaded areas indicate the gravid females, the unshaded the non-gravid.

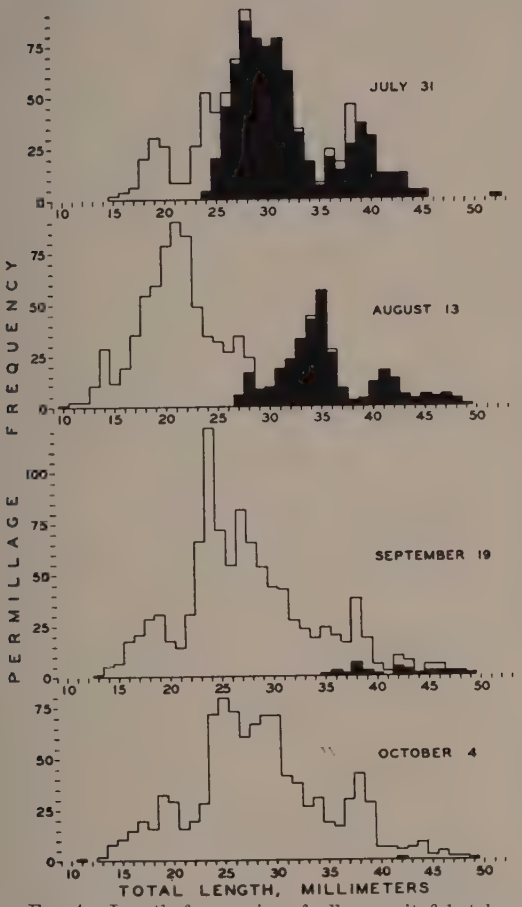

FIG. 4. Length frequencies of all mosquitofish taken in each of four collections from Parr's Pond, Macon County, Illinois, during 1940. The shaded areas indicate the gravid females, the unshaded the non-gravid.

Pond, in the last 4 samples from the Sanitary District Lake, and in all collections from Parr's Pond.

The numbers and size ranges of all females in each collection are given in Table 3 for the Argonne Woods Pond, Table 4 for the Sanitary District Lake, and Table 5 for Parr's Pond. The length frequencies are graphically represented in Figures 2, 3, and 4 respectively.

AGE AND GROWTH OF PARENT FISH

The parent stock of Gambusia in the Argonne Woods Pond grew more rapidly and to a larger size than did those in the Sanitary District Lake. The average size of the parent fish in the Argonne Woods Pond increased from 37.9 mm. on May 19 to 52.6 mm. on July 13, a growth of 14.7 mm. in a period of 55 days. The parent fish in the Sanitary District Lake grew much more slowly; from an average length of 38.1 mm. on June 9 to one of 40.4 mm. on September 14, an increase of only 2.3 mm. in 96 days. In the Argonne Woods Pond, the females reached a maximum length of 59 mm. (Table 3) by the middle of July in their second summer of life, after which time they began to die off rapidly (only a few attained a greater length than 56 mm.). The parent females in the Sanitary District Lake grew to a maximum length of 47 mm. (Table 4) but only relatively few attained a greater length than 43 mm. The numbers of these fish in the collections did not indicate any great mortality until after September 13 when they became scarcer and also appear to have stopped growing. Fair numbers of the parent stock lived through their second summer in the Sanitary District Lake, however, as they were present in the October collection. The presence of so many parent fish in October raises the interesting possibility of survival into the third summer. However, none of the fish taken early in the season from either of the ponds bore more than 1 annulus on their scales. As mentioned earlier, only one of the original fish stocked in Parr's Pond was taken in the collections. That fish was known to have been in its second summer of life (1 annulus). Collections made in Parr's Pond by Dr. George W. Bennett of the Illinois Natural History Survey the following spring (1941) failed to reveal any of the original stock.

Apparently the fish in the Argonne Woods Pond and Parr's Pond, having grown rather rapidly and

TABLE 3. Numbers of female mosquitofish, both parent and offspring, with minimum, average, and maximum total lengths in millimeters, in each of six collections from the Argonne Woods Pond, Cook County, Illinois, 1939.

Date of Collection	Offspring				Parents			
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
May 19	.....	..	....	..	39	33	37.9	45
June 9	1,321	10	17.4	23	74	43	48.8	59
July 13	1,367	11	26.4	41	215	46	52.6	58
August 14	2,214	12	26.5	44	4	50	51.8	55
September 13	371	14	25.3	43	..	..	....	..
October 19	624	16	29.9	46	..	..	...	..

TABLE 4. Numbers of female mosquitofish, both parent and offspring, with minimum, average, and maximum total lengths in millimeters, in each of six collections from the Sanitary District Lake, Cook County, Illinois, 1939.

Date of Collection	Offspring				Parents			
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
May 19	.....	..	....	..	2	31	31	31
June 9	.....	..	....	..	71	32	38.1	44
July 13	1,214	12	24.4	28	324	33	39.5	44
August 14	811	19	26.9	32	917	34	40.1	44
September 13	1,863	13	27.4	34	398	36	40.4	47
October 19	2,777	17	28.3	34	196	36	39.6	45

TABLE 5. Numbers of female mosquitofish, both parent and offspring, with minimum, average, and maximum total lengths in millimeters, in each of four collections from Parr's Pond, Macon County, Illinois, 1940. The single parent fish was from the original planting.

Date of Collection	Offspring				Parents			
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
July 31	494	15	29.8	45	1	52	52	52
August 13	1,042	10	25.6	49	..	..	..	..
September 19	920	13	27.1	49	..	..	..	..
October 4	1,144	11	28.3	49	..	..	..	..

to a larger maximum size as compared with those of the Sanitary District Lake, disappeared from the population very shortly after the end of the reproductive period, whereas those from the Sanitary District Lake, having grown more slowly and to a smaller maximum size, continued to live on for some time. In both cases, however, the parent fish did not survive to the third summer (2 annuli). The fact that the parent females in all of the ponds disappeared from the populations after their reproductive period regardless of their size and did not survive to their third summer of life leads to the belief that the maximum life span of these individuals seldom, if ever, extends over more than two summers.

In all of the collections of mosquitofish made by the writer, the largest female individual encountered was 63 mm. long. It was taken in her second summer of life from a small artificial pond in Oakland County, Michigan. A female *G. a. affinis* of similar length was the largest specimen in any of the collections of the Museum of Zoology at the University of Michigan.

Size and Growth of Offspring

The newly born young of Gambusia are usually 8-9 mm. long. Determination of growth of the offspring from the length-frequency data in Figures 2, 3, and 4 is made somewhat difficult by the presence of several broods of young that were born at different times during the summer. These broods of young, cast at rather regular intervals, reflect their growth to a certain extent by modes in their length frequencies. These modes, however, may be obscured in samples taken late in the summer because of variations in the periodicity at which broods are liberated and because individuals of the first brood of offspring, having become mature, give birth to offspring at intervals not necessarily coincident with those of the parent stock.

The first offspring in the Argonne Woods Pond were present in the collection of June 9. There was a rapid increase in the length of these individuals early in the season followed by a slower growth rate later on. From a length of 8-9 mm. at birth (about June 1), they exhibited a maximum increase in length of 14 mm. the first 9 days and within 43 days after birth had attained a maximum total length of 41 mm. However, in an additional 32 days (75 days after birth) the maximum size was increased to only 44 mm. Apparently this brood had begun to die off by the middle of August inasmuch as it was not manifest in the September or October collections. The actual rate of growth of the other broods is obscure.

No offspring were taken in the collections from the Sanitary District Lake until July 13. The first brood, probably born shortly after the middle of June (based on the stage of development of embryos carried by females in the June collection), did not grow as rapidly or attain so large a size as those in the Argonne Woods Pond. These more slowly growing individuals reached a maximum size of 28 mm. in the first 25-30 days of life (an increase in length of 19 mm.) and a month later had grown only an additional 4 mm. If the largest individuals of this first brood were still present at the time of the October collection, the maximum size could not have been greater than 34 mm.

The offspring in the population of Parr's Pond grew even more rapidly and to a greater length than those in the Argonne Woods Pond. The first brood was born about July 8 (based on an estimate of 49 days, from the time of stocking, for incubation and attainment of maturity—see page 15) and had attained a maximum length of 45 mm. by July 31—an increase of 36 mm. in 23 days. In the subsequent 13 days those fish grew an additional 4 mm. to a maximum size of 49 mm. Similarly as the fast-growing fish in the Argonne Woods Pond the individuals of the first brood in Parr's Pond began to disappear from the population by mid-August and were not present in the September and October collections.

Thus, individuals of the first brood of offspring in rapidly growing populations of *G. a. affinis* (Argonne Woods Pond, Parr's Pond) live only a few months and seldom, if ever, survive to the second summer of life, whereas, those of a more slowly growing population (Sanitary District Lake) tend to live longer and may survive to the second summer. In both the fast- and slow-growing populations, however, individuals of the broods subsequent to the first, although they grow just as rapidly and attain as large a size as those of the first brood, apparently survive the winter and are present in the population of the following year as the parent stock.

Size and Age at First Maturity and Length of the Reproductive Period in Females

In a fish that is as promiscuous in its breeding habits as Gambusia there is little chance that copulation will not take place as soon as the female is physiologically ready to bear young. Hence the presence of fertilized ova is the best available macroscopic indication for the attainment of maturity in female mosquitofish. The age and size at first maturity are dependent on the time of year when the female offspring are born and on the rate of growth. In all

of the ponds observed in this study there was an abundance of mature male mosquitofish.

The numbers of gravid and non-gravid female mosquitofish which had attained a length as great as that of the smallest gravid female taken in the collections from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond are given in Tables 6, 7, and 8 respectively. All females taken in the May and June collections from the Argonne Woods Pond and the Sanitary District Lake were gravid and had obviously survived from the preceding summer. No samples were collected from Parr's Pond until July 31. Gravid young-of-the-year females were first taken in the July collections from all 3 ponds.

The smallest gravid mosquitofish was taken from the Argonne Woods Pond on July 13. It was 31 mm. long (Table 6) and was probably not more than 6 weeks old (see page 11). Among the other young of the year in the July collection, the fish that had reached a length of 33 mm. were predominantly gravid and all those 35 mm. or more in length carried eyed embryos. Inasmuch as the smallest gravid female carried eyed embryos about midway in their development, it is apparent that they had reached maturity at an earlier date and probably were considerably smaller when they first became mature. In the August collection from the Argonne Woods Pond only a part of the female offspring whose lengths were 41 mm. or greater were gravid and none of the many individuals between 31 and 41 mm. long carried young. Also one of the parent fish (50 mm. long) taken in that collection was not gravid. No gravid fish were collected in September or October.

In the Sanitary District Lake (Table 7), the smallest gravid female was 26 mm. long. It was collected on July 13. Along with 8 others it carried embryos that had not yet reached the eyed stage. None of the young of the year taken in this lake on July 13 carried eyed embryos. These young, probably 4-5 weeks old, were either missed in the collection of June 9 or were born very shortly thereafter. There was no sharp demarcation between the lengths of the gravid and non-gravid females in the July or August collections.

The smallest gravid females in the collections from Parr's Pond were 24 mm. long (Table 8). In all probability they were members of the second brood of offspring liberated by individuals of the original stock (see page 12) and were not more than 4 weeks old. In the July collection the females that were 26 mm. or more in length were predominantly gravid and all but 21 of the 276 females 29 mm. long or longer were gravid. In the August collection those that had reached a length of 28 mm. or more were predominantly gravid and only 8 of the 316 individuals 30 mm. or more in length did not carry young. In that sample all females that were 39 mm. long or longer carried young. In Parr's Pond, but in none of the other Illinois ponds, a few of the females taken during September and October were gravid.

TABLE 6. Numbers of gravid and non-gravid female mosquitofish which had attained the length of the smallest gravid female (31 mm.) taken in six collections from the Argonne Woods Pond, Cook County, Illinois, 1939. No non-gravid females were taken in the first two collections and no gravid females were taken in the last two.

Total length (millimeters)	May 19 Gravid	June 9 Gravid	July 13 Gravid	July 13 Non-gravid	August 14 Gravid	August 14 Non-gravid	September 13 Non-gravid	October 19 Non-gravid
31	..	..	1	74	..	105	13	41
32	..	..	22	44	..	58	17	37
33	1	..	30	16	..	53	16	20
34	2	..	48	7	..	70	11	38
35	5	..	54	..	..	76	6	39
36	8	..	38	..	..	71	12	17
37	4	..	25	..	..	31	2	14
38	2	..	18	..	..	20	3	22
39	6	..	14	..	..	13	3	20
40	6	..	6	..	..	5	2	18
41	1	..	3	..	4	9	..	9
42	1	..	..	..	1	3	..	2
43	..	1	..	..	4	5	2	..
44	..	2	..	..	1	1	..	..
45	3	7	.	.	..	..	..	..
46	..	8	1	..	..	..	..	1
47	..	8	.	.	..	..	.	..
48	..	14	2	.	.	.	.	..
49	..	6	12	.	.	.	.	..
50	..	9	10	..	1	1	..	..
51	..	5	30	.	..	.	.	..
52	..	5	54	..	1	..	..	..
53	..	4	46	..	..	.	.	..
54	..	3	28	.	.	.	..	..
55	..	1	11	..	1	.	.	..
56	..	..	15	.	..	.	.	..
57	..	..	5	.	.	..	.	..
58	..	..	1	.	.	.	..	..
59	..	1	.	.	..	.	.	..
Total	39	74	474	141	13	521	87	278

In the collections from the Argonne Woods Pond and the Sanitary District Lake single modes in the length-frequency distributions indicate that only the first brood of offspring reached maturity during the first summer of life. In comparable data from Parr's Pond, however, there are 2 apparent modes in the length frequencies of each of the first 3 collections, indicating that not only the first brood, but the second as well, reproduced during the first summer. In all ponds the first offspring were born before July 13 and their age at first maturity was 4-5 weeks. Further, these broods, as reflected by their numbers in succeeding collections, were rapidly disappearing from the population by late summer. Thus it may be assumed that, in Illinois, mosquitofish which become gravid during their first summer of life do not survive to the following summer and the length of their reproductive period is limited to 8 or 10 weeks

On the other hand, non-gravid offspring as large or larger than the gravid individuals were present in the populations later in the year. It is not known why the female offspring taken in August (second brood in the Argonne Woods Pond and Sanitary District Lake; third brood in Parr's Pond) were not gravid, although they were as old and as large as

TABLE 7. Numbers of gravid and non-gravid female mosquitofish which had attained the length of the smallest gravid female (26 mm.) taken in six collections from the Sanitary District Lake, Cook County, Illinois, 1939. No non-gravid females were taken in the first two collections and no gravid females were taken in the last two.

Total length (millimeters)	May 19 Gravid	June 9 Gravid	July 13 Gravid	July 13 Non-gravid	August 14 Gravid	August 14 Non-gravid	September 13 Non-gravid	October 19 Non-gravid
26	...	...	1	314	...	67	292	213
27	...	...	6	120	...	105	236	359
28	...	...	2	22	1	141	209	329
29	...	...	...	...	2	142	243	305
30	...	...	...	...	6	71	298	467
31	2	...	...	...	6	23	175	499
32	...	1	...	...	...	2	34	195
33	...	...	...	4	...	...	7	19
34	...	3	...	5	1	...	1	3
35	...	2	1	11	1	1	...	...
36	...	8	1	10	2	3	5	2
37	...	9	6	10	...	8	9	4
38	...	15	19	12	11	46	25	33
39	...	22	39	20	57	134	60	57
40	...	6	66	7	145	163	107	63
41	...	3	54	8	156	88	118	16
42	...	...	38	...	68	21	47	18
43	...	1	7	1	9	...	22	2
44	...	1	5	...	3	...	2	...
45	...	...	...	...	...	...	1	1
46	...	...	...	...	...	...	1	...
47	...	...	...	...	...	...	1	...
Total	2	71	245	544	468	1,025	1,893	2,585

the gravid young-of-the-year females collected in July. Larger offspring (earlier broods) collected at the same time were gravid. If ample time remained for those older offspring to bear a brood of young certainly time must have been sufficient to allow the younger ones to follow suit. Perhaps the younger ones, although of a size and age comparable to the older individuals when they bore their first brood, had not become physiologically ready to bear young. The fact remains that individuals of the later broods (born after mid-July) did not become gravid but rather survived the winter and were present at the beginning of the following season as the parent stock.

As previously stated, the parent fish, when taken in the May collections from the Argonne Woods Pond and Sanitary District Lake, were gravid. These fish, having been born sometime after mid-July of the preceding year, were 8-10 months old when they first became gravid. They continued to bear young until they disappeared from the population during July and August. The numbers of these fish, as reflected by the length-frequency distributions in the first 4 collections, had disappeared by September. Having been gravid since early May and, in some cases, continuing to bear young until late August these individuals exhibited a reproductive period of about 14-15 weeks.

Sokolov (1936) recorded an average growth among adults of Gambusia from 34 mm. on June 9 to 42 mm. on August 27, an increase in length of 8 mm. in 79 days. Among the offspring he found that growth proceeded from 7.6 mm. at birth to 23.1 mm. at an age of 37 days, an increase of 15.5 mm. Further, Sokolov maintained that the offspring reached puberty within 36-40 days after birth but that the peak of fecundity was not reached until the fish attained a length of 45-50 mm., a size that corresponded to an age of 2-3 years as shown by an analysis of their scales. The largest fish attained a length of 50-55 mm. The fish referred to by Solokov had been introduced into rice fields in Turkestan as a mosquito-control measure. Sella (1929) found that only a small number of mosquitofish attained an age of 2 years when living under "natural conditions" in stock ponds in central Italy. Regarding the question of maximum age among mosquitofish under natural conditions, the writer is in agreement with Sella inasmuch as no fish taken from the Argonne Woods Pond or the Sanitary District Lake, where populations of Gambusia had maintained themselves for several years previous to the time of collection, had entered their third year summer of life (2 annuli). Sokolov, in admitting that mosquitofish reached maturity when 36-40 days old and then maintaining that they did not reach their peak fecundity until an age of 2-3

TABLE 8. Numbers of gravid and non-gravid female mosquitofish which had attained the length of the smallest gravid female (24 mm.) taken in four collections from Parr's Pond, Macon County, Illinois, 1940.

Total length (millimeters)	July 31 Gravid	July 31 Non-gravid	August 13 Gravid	August 13 Non-gravid	September 19 Gravid	September 19 Non-gravid	October 4 Gravid	October 4 Non-gravid
24	2	24	..	36	..	112	..	82
25	10	11	..	33	..	66	..	91
26	23	3	..	28	..	50	..	83
27	33	1	6	30	..	75	..	69
28	43	3	17	8	..	60	..	76
29	39	..	9	2	..	49	..	81
30	37	..	10	..	..	40	..	81
31	39	..	19	..	..	39	..	47
32	31	..	23	1	..	25	..	43
33	16	1	34	..	..	21	..	30
34	9	..	44	2	..	17	..	35
35	3	1	59	..	1	21	..	21
36	10	2	27	2	2	16	..	19
37	7	2	9	..	1	14	..	34
38	13	10	3	3	6	29	..	48
39	15	3	4	..	2	15	..	32
40	13	2	12	..	1	4	..	7
41	6	..	18	..	..	..	..	7
42	6	..	14	..	4	5	1	5
43	6	..	6	..	3	4	..	7
44	2	..	4	..	1	1	..	10
45	2	..	6	..	2	3	..	3
46	..	..	5	..	2	3	..	5
47	..	..	6	..	..	2	..	2
48	..	..	4	..	..	2	..	2
49	..	..	1	..	..	1	..	1
50	..	..	..	..	..	..	..	..
51	..	..	..	..	..	..	..	..
52	1	..	..	..	..	..	..	..
Total	366	63	340	145	25	674	1	921

years, inferred that the rate of growth between those ages was extremely slow. After having reached a length of 23 mm. in 37 days it required an additional 2 years for them to double their length and reach the height of the reproductive period. The fish in the Sanitary District Lake, which grew almost as slowly as those referred to by Sokolov, reached their peak fecundity at a smaller size (about 40 mm.) but apparently did not survive until their third summer. It may be that the individuals in the rice fields of Turkestan, having grown more slowly, lived to a greater age than those in any of the Illinois ponds.

Zanco (1933) reported having kept an individual of *G. holbrookii* living under aquarium conditions to an age of at least 5 years. The writer has kept individual mosquitofish in aquaria for nearly 4 years. Such longevity is probably to be attributed to the fact that the life span of many animals may be considerably prolonged when kept under conditions of semi-starvation.

Gestation Period

Information on the period of gestation was obtained by observing Gambusia in aquaria. Four adult female mosquitofish were placed in aquaria with mature males and as soon as copulation had taken place the females were separated from the males and placed in individual aquaria. These fish were fed daily and the aquaria were kept at room temperature. The incubation period ranged from 21 to 28 days (Table 9) and the average of the 14 gestation periods was 23.9 days. It should be mentioned here that some of the broods contained young that were slightly premature, that is, the yolk sac was not completely absorbed.

That the period of gestation under natural conditions is approximately the same as in the aquarium experiment is indicated by observations made in Parr's Pond during the summer of 1940. The patent vent and the still slightly distended body cavity indicated that some of the young-of-the-year female mosquitofish taken on July 31 had released a brood of young very recently. Here, the third generation made its appearance within 71 days after the pond was stocked. During that time the virgin females originally placed in the pond were fertilized, liberated a brood of young, which in turn reached maturity, were fertilized, and gave birth to a brood. Thus, the 71-day period is to be divided into 2 periods of gestation and the period required for the first brood to reach maturity. Using a figure near the minimum observed period of gestation and assuming that the duration of both incubation periods was the same and that the time required for the young to reach maturity was slightly under 4 weeks, a division of the 71-day interval into 2 gestation periods of 22 days each and a lapse of 27 days for the young to reach maturity seems a reasonable approximation. It has been shown previously that mosquitofish usually grow much faster under such favorable natural conditions as those in Parr's Pond than they do in aquaria.

Seale (1917) recorded periods of gestation for 5 separate broods of young liberated by a single mosquitofish between December 8, 1915 and June 23, 1916. The duration of those periods ranged from 21 to 79 days, with an average of 39.6 days. The longest incubation period is far out of line with the other 4, which averaged 29.8 days. The shortest period of gestation was the same as that observed in the present study but that of the longest was more than 3 times as great.

In observing the parturitions of 29 female mosquitofish living under conditions simulating those of nature, Dulzetto (1928, 1934, 1935) found that the 49 intervals between broods ranged from 19 to 61 days with an average of 27.7 days. The longest period, which occurred between the first and second broods liberated by a female during the summer of 1933, was referred to as abnormal.

Working with *G. a. holbrookii* in the region of Moscow, Samokhvalova (1941), found that, whereas the size of the aquaria, the temperature of the water, and the number of fish per unit of volume of water have an effect on the growth of that poeciliid as well as all other fish, those factors were not decisive in causing the under-development of aquarium-reared Gambusia. The decisive factor was held to be direct sunlight. When the aquaria were placed in the direct sunlight, the mosquitofish grew well and began to spawn. When moved back to ordinary laboratory conditions, the fish in the aquaria continued to grow for a short period of time and then apparently relapsed to subnormal growth and reproduction. It was concluded that sunlight is one of the chief prerequisites for the normal growth and spawning capac-

TABLE 9. The date of fertilization, date of birth of each brood, and number of days in the periods of gestation for each brood liberated by four female mosquitofish in laboratory aquaria, 1940.

Female number	Date of fertilization	First Brood		Second Brood		Third Brood		Fourth Brood	
		Date of birth	Days of incubation	Date of birth	Days of incubation	Date of birth	Days of incubation	Date of birth	Days of incubation
1	Aug. 2	Aug. 27	25	Sep. 20	24	Oct. 11	21	.....	..
2	Aug. 2	Aug. 25	23	Sep. 16	22	Oct. 10	24	Nov. 1	22
3	Aug. 2	Aug. 27	25	Sep. 19	23	Oct. 12	23	Nov. 2	21
4	Aug. 2	Aug. 30	28	Sep. 25	26	Oct. 22	27	.....	..
Average			25.2		23.8		23.8		21.5

ity of mosquitofish under either laboratory or natural conditions.

Gambusia is one of the poeciliids that does not exhibit superfetation (Turner 1937). One brood of young is born before any other ova have matured sufficiently to be fertilized. There is no overlapping of broods. Consequently only a single brood of developing embryos is present in the ovary at any one time and the embryos in that brood are in a similar stage of development. The studies of Kuntz (1913) and especially those of Turner (1937) have adequately outlined the reproductive cycle of Gambusia.

The manner in which the young of Gambusia are born was observed while collecting data on the duration of the period of gestation. All young were liberated individually. Some were born headfirst, some tailfirst, and some sideways and they were always more or less ball-like when released. Parturition of a single brood required from 23 to 67 minutes. Sometimes a single offspring was released and then, after an interval of several minutes, 3 or 4 were liberated in rapid succession, followed by another time interval and the release of more young. The greatest number of young observed to be released in rapid succession was 6, born in an interval of 3 seconds. The birth of a brood on August 27, 1940, was annotated as follows: ". . . She was seen to give birth to 23 young of which 9 were eaten immediately upon being liberated, the remaining 14 young were removed from the tank. Then the female gave birth to 9 more young in groups as follows: 1, 3, 2, 2, 1, which were devoured as soon as each group was released. Ten other young born later were rescued from the aquarium and placed with those which had been removed previously. . . ." The interval of time required for the birth of the 42 young was 52 minutes. Perhaps the removal of some of the young from the aquarium either lengthened or shortened the process to some degree. As soon as the young were born they sank to the bottom of the aquarium where they remained for several hours, rising to the surface at intervals. Within 5 hours after birth all of the young were actively swimming and feeding.

Ryder (1885) reported the observations of Mr. A. A. Duly on the manner in which Gambusia are born:

> "The young when born are stated by Mr. Duly to be about three-eighths of an inch in length, and to be expelled in a single mass, consisting of eight to eleven young fishes at a single effort. This mass as soon as it escapes is seen to be composed of infant Gambusia, which at once separate and swim away. . . . My informant also tells me that the parent fishes devoured their young as soon as they were born if they were not separated, by transferring one or the other at once to another aquarium. . . . He also noticed that more than one brood seemed to be produced by the same parent consecutively and during the same season. . . ."

The birth of the young as described by Mr. Duly does not conform with observations made during the present study. Gambusia were never observed to be born in masses of several individuals but were always liberated singly though often released in rapid succession. The young at birth, when rounded up, are 2-3 mm. in diameter and it would be physically impossible for any adult female mosquitofish to expel a mass of 8-11 offspring at a single effort. Figure 5 shows a gravid female mosquitofish with her brood of 79 embryos which had been removed prior to birth. These embryos were fully developed and nearly ready to be released. Figure 6 shows an adult male and female mosquitofish together with some newly born young.

FIG. 5. Gravid female of Gambusia and brood of 79 eyed embryos. These embryos are nearly fully developed, the yolk sac having been completely absorbed, and are about ready for birth.

FIG. 6. Adult male and female of Gambusia together with young (the female is not the parent). The young are about four hours old.

FECUNDITY

The numbers of ova produced by various species of teleost fishes are roughly proportional, inversely, to the life expectancy, which to a large degree is determined by the extent of parental care. Loss at any immature stage needs to be compensated for by greater fecundity. In some fishes, as the cod and pollock, several million eggs may be shed by a single female, and a number of fresh-water species are known to deposit many thousands of ova at each spawning. Although predation and other factors lead to the destruction of enormous number of eggs, at least enough ova produce young which reach maturity to insure maintenance of the population. Gambusia and other Poeciliidae contrast with fishes that produce thousands or millions of unprotected eggs in that they are viviparous and the number of young in a brood rarely exceeds 300. The newborn fish, having been protected from many of the dangers that beset the larvae of oviparous fishes, are at an advanced stage of development and, being very active, begin to fend for themselves immediately. Thus, although the number of individual mosquitofish in a single brood is very small as compared with the number of eggs deposited by most oviparous fishes, nearly all of the ova fertilized in the poecillids become well-developed young. This viviparity coupled with the release of several broods during each season tends to assure the perpetuation of the race.

The present study shows that the number of young produced by a single female mosquitofish depends on the number of broods liberated during a season, the length of the mother fish, the time of season at which the individual broods are cast, and the locality in which the mother fish lives. These factors are interdependent. The number of young carried by a female of a given size in a fertile pond is larger than the brood carried by a female of the same size in a pond that is not so productive. Similarly, the size of the brood carried by a female of a given length at the height of the reproductive season is larger than that carried by a female of the same length at a later date.

The marked correlation between the brood size and the length of the mother fish and the differences with locality were determined by an analysis of the collections that were made from the various ponds during the summers of 1938-1940. There were wide differences in the sizes of the broods carried by females in the 5 ponds near Chicago in 1938. Furthermore, the data from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond showed that the differences were manifest in successive collections taken throughout the summer. The first 2 ponds from which successive collections were made supported standing populations of mosquitofish and were considerably different in type of habitat and natural fertility. Parr's Pond, on the other hand, supported a newly established population and, due to the effects of the continuous deposition of excreta on the surrounding terrain by cattle and hogs pastured in the area, was probably more fertile than either of the other ponds.

The information on the fecundity of *Gambusia a. affinis* presented here is based on actual counts of 2,380 individual broods that yielded a total of 114,311 eyed embryos. The largest number of embryos in one brood, 315, was carried by a 59 mm. female of the parent stock taken from the Argonne Woods Pond on June 9. The smallest brood carried by female of that stock was one of 10 embryos taken from a specimen 35 mm. long from the Sanitary District Lake. Among the young of the year the largest brood, 218 embryos, was taken from a 47 mm. female from Parr's Pond on August 13, and a single embryo from a 34 mm. fish taken in the Sanitary District Lake on August 14 comprised the smallest brood.

NUMBERS OF BROODS LIBERATED BY A FEMALE DURING A SEASON

It has been previously pointed out (page 12) that the reproductive period in female mosquitofish varies from 8 to 15 weeks, depending primarily on the age at first maturity. Females that mature during the first summer of life usually reproduce over a period of only 8 or 10 weeks, whereas fish that do not reach maturity until their second summer enjoy a reproductive period of 14-15 weeks. Also, it has been shown (page 14) that the average gestation period is about 23-24 days. A fish that reproduces for 8 to 10 weeks liberates 2 or possibly 3 broods, whereas one that continues to reproduce for 14 to 15 weeks gives birth to 4 and possibly 5 broods.

The presence of gravid parent females in the first 4 collections from the Argonne Woods Pond (Table 6) and the Sanitary District Lake (Table 7) indicates that, in each of those populations, the fish which became gravid for the first time during their second summer of life probably bore 4 broods during the season. However, the graphic representations of the length frequencies in Figures 2 and 3 indicate that, in the Argonne Woods Pond, the parent stock liberated at least 4 and possibly 5 broods during the season whereas those in the less productive Sanitary District Lake usually gave birth to 3 broods and never had more than 4. It is evident that the fish in the more productive pond produced a greater number of broods than did those from the Sanitary District Lake. In Parr's Pond (Fig. 4) it appears that the parent stock gave birth to at least 4 broods of young.

Among the offspring in the 3 ponds, the data from the same tables and figures show that only the first brood of young in the Argonne Woods Pond and the Sanitary District Lake reached maturity during the first summer and that in each case they liberated no more than 2 broods during the season. In Parr's Pond, on the other hand, the first 2 broods of offspring reached maturity during their first summer of life and some individuals continued to reproduce until October. Although the data are not conclusive, it seems probable that the first brood, in that newly

established population, liberated at least 3 broods of young and that the second brood liberated 2 and possibly 3 broods.

From the foregoing discussion it appears that, in Illinois, parent mosquitofish liberate an average of 4 broods of young per season and that the offspring, which mature during their first summer of life, give birth to 2 and possibly 3 broods.

Relationship between the Length of the Gravid Female and the Number of Young per Brood

In general, as was to be expected, the average number of young carried by females of a given size in a single collection was found to increase with the length of the mother fish, in both offspring and parent stocks, in all populations of mosquitofish, throughout the reproductive period. Of the 17 collections taken during the present study (Tables 10-13) the data from 15 of the samples showed increases in the average brood size with the increased length of the mother fish. In each of the other 2 samples the similar lengths of the few gravid females precluded comparison with fish of other sizes. There was some variability in the relationship between the size of the individual mother fish and the number of young when mother fish of similar lengths in the same population were compared, and also there were some discrepancies in the regular increase of the average brood size of females of increasing lengths. Most of those irregularities, however, were traceable to the small numbers of gravid females of certain sizes in the collections.

At first glance the determining factor in the relationship between the length of the mother fish and the number of young per brood appears to be the physical limitation of size in the gravid female; a small fish does not provide as much space for carrying embryos as does a larger one. If space alone were the determining factor, the number of young would increase approximately in direct proportion to the cube of the length of the mother fish. If that relation held the result would be a regularly increasing number of young per brood as the parent female continued to grow and reproduce. The number of embryos per brood, however, does not increase regularly in that proportion. Furthermore, the rate of increase in the size of the brood per unit length of the gravid female is not constant for all sizes of mosquitofish. Obviously the space provided by the mother is not the sole factor that determines the number of young in a brood.

The data given in Tables 10-13 were rectified by

Table 10. Average numbers of eyed embryos taken from female mosquitofish of different lengths in collections from five ponds in the Forest Preserve District of Cook County, Illinois, August 1938.

Total length (millimeters)	Pond 1		Pond 2		Pond 3		Pond 4		Pond 5	
	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos
26	2	21	9	12	..	..	..	..	..	..
27	3	27	9	16	1	9	..	..	..	..
28	1	25	3	15	2	26	1	15	..	..
29	1	35	4	13	..	..	5	16	..	..
30	..	..	3	14	2	32	1	21	..	..
31	..	..	4	17	1	65	3	28	1	14
32	..	..	3	22	5	49	2	36	..	..
33	..	..	8	20	4	62	..	..	1	9
34	..	..	21	24	7	66	..	..	2	9
35	..	..	28	31	7	64	1	50	3	16
36	..	..	21	37	4	79	..	..	..	..
37	..	..	7	38	3	71	1	56	1	24
38	..	..	2	43	1	75	7	56	4	25
39	..	..	2	60	..	..	..	..	1	23
40	..	..	7	58	1	49	1	81	9	39
41	..	..	10	71	1	54	..	..	6	54
42	..	..	7	60	..	..	2	68	4	55
43	..	..	1	49	..	..	..	..	3	64
44	..	..	..	..	1	126	..	..	4	87
45	..	..	..	..	2	122	..	..	..	..
46	..	..	..	..	..	..	1	66	3	92
47	..	..	..	..	..	..	..	..	3	88
48	..	..	..	..	..	..	..	..	2	109
54	..	..	..	..	..	..	..	..	1	174
57	..	..	..	..	..	..	..	..	1	123
Total	7	183	149	4,891	42	2,650	25	1,053	49	2,726
Average	27.1	26.1	34.5	32.8	34.7	63.6	34.7	42.1	41.4	55.6
Percentage of gravid females in total	6.7	...	31.5	...	67.7	...	86.2	...	62.8	...

TABLE 11. Average numbers of eyed embryos taken from female mosquitofish of different lengths in each of four collections from the Argonne Woods Pond, Cook County, Illinois, 1939.

Total length (millimeters)	MAY 19		JUNE 9		JULY 13		AUGUST 14	
	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos
31	..	..	..	...	1	8	..	..
32	..	..	..	...	22	15	..	..
33	1	31	..	...	30	21	..	..
34	2	46	..	...	48	24	..	..
35	5	50	..	...	54	29	..	..
36	8	62	..	...	38	37	..	..
37	4	62	..	...	25	43	..	..
38	2	64	..	...	18	54	..	..
39	6	62	..	...	14	65	..	..
40	6	67	..	...	6	86	..	..
41	1	73	..	...	3	107	4	16
42	1	60	..	...	..	...	1	9
43	..	..	1	172	..	...	4	15
44	..	..	2	151	..	...	1	23
45	3	78	7	166	..	...	..	..
46	..	..	8	201	1	136	..	..
47	..	..	8	199	..	...	..	..
48	..	..	14	206	2	146	..	..
49	..	..	6	216	12	150	..	..
50	..	..	9	207	10	138	1	49
51	..	..	5	197	30	136	..	..
52	..	..	5	232	54	155	1	30
53	..	..	4	227	46	156	..	..
54	..	..	3	238	28	159	..	..
55	..	..	1	244	11	180	1	49
56	..	..	..	...	15	184	..	..
57	..	..	..	...	5	191	..	..
58	..	..	..	..	1	99	..	..
59	..	..	1	315	..	...	..	..
Offspring:								
Total	..	..	..	...	259	8,886	10	156
Average length	..	..	..	...	35.3	...	42.2	...
Average number	..	..	..	...	..	34.3	..	15.6
Parent stock:								
Total	39	2,383	74	15,201	215	33,480	3	128
Average length	37.9	...	48.8	...	52.6	...	52.3	...
Average number	...	61.1	...	205.4	...	155.7	...	42.7

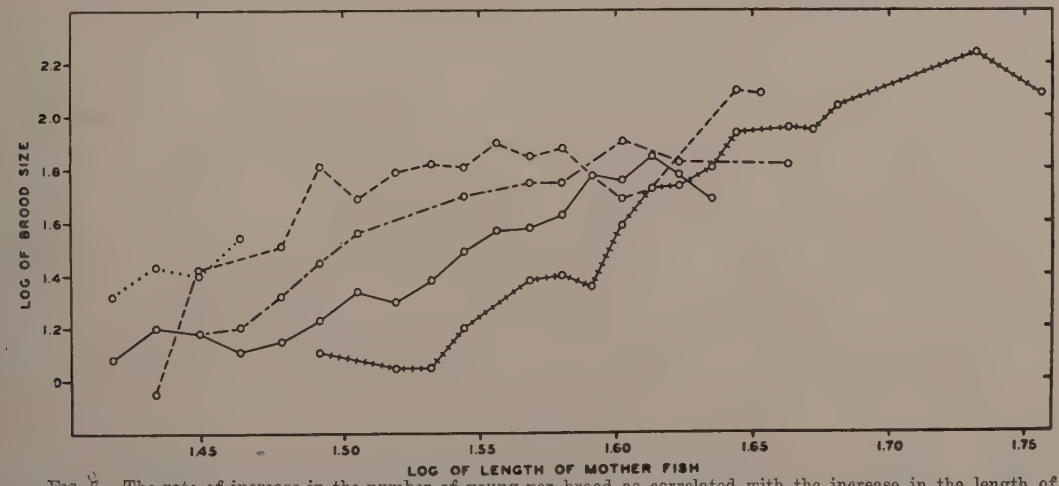

FIG. 7. The rate of increase in the number of young per brood as correlated with the increase in the length of the mother fish in collections from five ponds in the Forest Preserve District of Cook County, Illinois, August 1938. These curves represent the data in Table 10 which have been rectified by plotting the logarithm of the size of the brood against the logarithm of the length of the mother fish. Collections are indicated as follows: Pond 1, ... ; Pond 2, ————; Pond 3, - - - - - ; Pond 4, - —— - —— ; Pond 5, -|-|-|-

TABLE 12. Average numbers of eyed embryos taken from female mosquitofish of different lengths in each of four collections from the Sanitary District Lake, Cook County, Illinois, 1939.

Total length (millimeters)	MAY 19		JUNE 9		JULY 13		AUGUST 14	
	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos
28	..	..	..	..	..	..	1	10
29	..	..	..	..	..	..	2	8
30	..	..	..	..	..	..	6	4
31	2	19	..	..	..	..	6	5
32	..	..	1	22	..	..	..	..
33	..	..	..	..	..	..	..	..
34	..	..	3	27	..	..	1	1
35	..	..	2	22	1	10	1	4
36	..	..	8	24	1	19	2	4
37	..	..	9	24	6	19	..	..
38	..	..	15	32	19	20	11	7
39	..	..	22	30	39	24	57	7
40	..	..	6	37	66	25	145	8
41	..	..	3	43	54	28	156	9
42	..	..	..	..	38	38	68	11
43	..	..	1	48	7	52	9	14
44	..	..	1	63	5	55	3	16
Offspring:								
Total	..	..	..	..	..	..	15	80
Average length	..	..	..	..	..	..	30.1	..
Average number	..	..	..	..	..	..	..	5.3
Parent Stock:								
Total	2	38	71	2,157	236	6,704	453	3,975
Average length	31	...	38.8	...	40.3	...	40.5	...
Average number	...	19	...	30.4	...	28.4	...	8.8

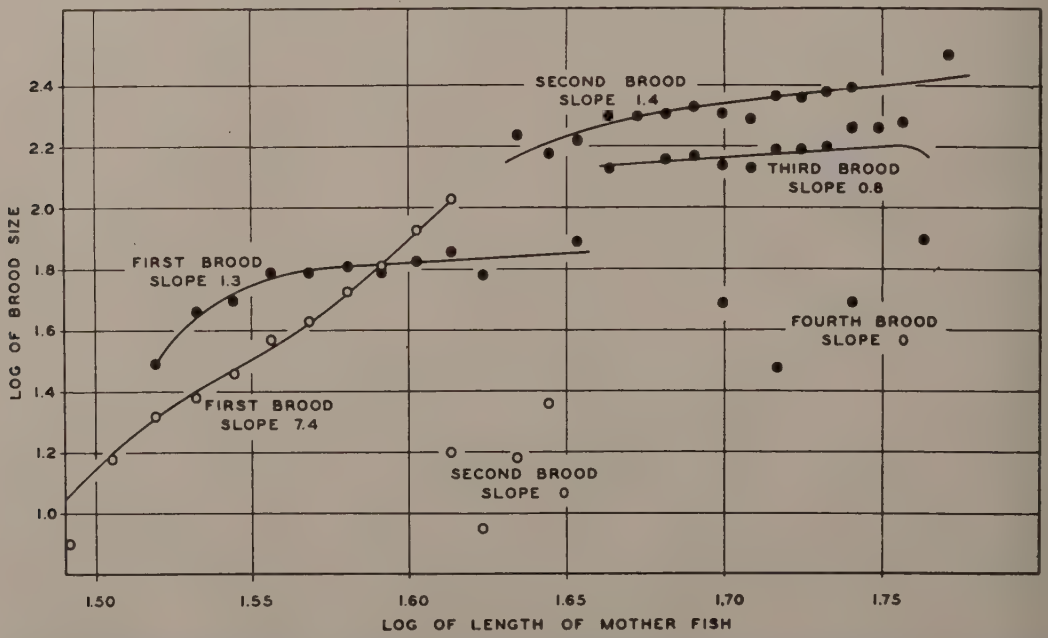

FIG. 8. The rate of increase in the size of the brood as correlated with the increase in the length of the mother fish in successive collections from the Argonne Woods Pond, Cook County, Illinois, during 1939, as given in Table 11. The slope values represent the exponent of the rate of increase as indicated by a straight line drawn through the main part of each curve. The solid dots represent the parent females, the open circles the gravid offspring.

TABLE 13. Average numbers of eyed embryos taken from female mosquitofish of different lengths in each of four collections from Parr's Pond, Macon County, Illinois, 1940.

Total length (millimeters)	JULY 31		AUGUST 13		SEPTEMBER 19		OCTOBER 4	
	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos	Number of gravid females	Average number of eyed embryos
24	2	9	..	...	..	..	..	..
25	10	18	..	...	..	..	..	..
26	23	18	..	...	..	..	..	..
27	33	23	6	10	..	..	..	..
28	43	27	17	8	..	..	..	..
29	39	31	9	12	..	..	..	..
30	37	36	10	15	..	..	..	..
31	39	42	19	17	..	..	..	..
32	31	48	23	20	..	..	..	..
33	16	52	34	23	..	..	..	..
34	9	58	44	28	..	..	..	..
35	3	49	59	31	1	4	..	..
36	10	62	27	34	2	10	..	..
37	7	81	9	40	1	23	..	..
38	13	72	3	63	6	10	..	..
39	15	80	4	74	2	9	..	..
40	13	83	12	67	1	4	..	..
41	6	78	18	67	..	..	..	..
42	6	95	14	69	4	6	1	31
43	6	103	6	69	3	14	..	..
44	2	98	4	71	1	14	..	..
45	2	149	6	100	2	56	..	..
46	..	..	5	95	2	52	..	..
47	..	..	6	118	..	..	..	..
48	..	..	4	102	..	..	..	..
49	..	..	1	98	..	..	..	..
52	1*	154	..	...	..	..	..	..
Total	365	16,252	340	12,755	25	428	1	31
Average length	31.6	...	35.4	...	40.5	...	42.0	...
Average number	...	44.5	...	37.5	...	17.1	...	31.0

* From original planting, not included in computation of totals or means.

plotting the logarithm of the average number of embryos per brood against the logarithm of the length of the mother fish (Figs. 7-10). Here the slope of the curve is equivalent to the exponent to which the length of the mother fish is raised in the equation:

Number of embryos = length^n of mother fish.

The rectified curves indicate that the greatest rate of increase in the size of the brood as compared with the length of the mother occurred in the first brood produced by the gravid offspring taken in the July 13 collection from the Argonne Woods Pond (Fig. 8). That curve shows that the size of the brood varied as the 7.4 power of the length of the mother fish. Such a rate of increase in a biological relationship is most spectacular and were it to continue, there would result an increase of more than one hundred fold in the number of young with a doubling of the length of the mother. That, of course, would be a physical impossibility. Since the size of the embryos at birth is similar regardless of the size of the mother, the larger fish would have to carry more young than the increase in length would allow and the bellies of such fish would become so distended that they would rupture. Somewhere along the line such a relationship would have to break down as indeed it does. In the larger fish the fecundity increases at a drastically reduced rate. Even then some of the gravid parent females taken during this investigation were so swollen with young that locomotion was seriously impaired. In those fish the number of young varied only as the 1.4 power of the length of the mother.

For the ponds from which periodic collections were made during the reproductive season, the rectified data indicate that the rate of increase in the relationship between the size of the brood and the length of the mother fish was not the same for the various broods liberated during the season (regardless of the category of the mother fish). Nor was the rate of increase constant within any particular brood produced by females of different lengths within the same category. In most curves the slope was greatest for the second brood born to the mother. Among the parent fish from the Argonne Woods Pond (Fig. 8) the power of increase was approximately 1.3 for the first brood of young, increased slightly to 1.4 for the second brood, and then fell off to 0.8 for the third brood. In the Sanitary District Lake (Fig. 9) the slope of the curve for the first brood of young carried by the parent fish was 3.7, then rose to 5.5 for the second brood, and dropped to 4.2 in the third brood.

In Parr's Pond (Fig. 10), from which there were no adequate data on the fecundity of the parent fish, at least 2 broods of offspring reproduced during their first summer of life. The first brood of young produced by the first mature offspring had been liberated prior to July 31 (see page 15) and it is impossible to say what the relationship between the size of the brood and the length of the mother fish might have been. However, the size of the second brood varied as the 2.3 power of the length of the mother fish and that of the third brood varied as the 1.7 power. Among the individuals of the second brood of mature offspring the size of the first brood increased as the 4.1 power of the mother's length and that of the second brood increased as the 4.8 power. The curves for the fourth brood carried by the first mature offspring and the third released by the second brood of mature offspring in Parr's Pond indicate that for those lots the relationship has broken down completely.

From these data it may be inferred that, with the exception of the gravid offspring in the Argonne Woods Pond, the rate of increase in the size of the brood was generally greatest in the second brood of young liberated by all mother fish regardless of their size or age. Further, the actual numbers of embryos in the second brood carried by mother fish, both parent and offspring, of the various lengths in all ponds were greater than those in any preceding or subsequent broods.

Within a single brood produced by the mother fish of either category the slopes of individual curves changed with the increased length of the female. In the rapidly growing populations of Parr's Pond and the Argonne Woods Pond, with the single exception of the gravid offspring in the latter pond, the rate of increase gradually diminished as the length of the mother fish became greater. Such a decrease in the rate of increase held in both offspring and parent stocks, except as noted, throughout the summer in both ponds. Among the parent fish from the Argonne Woods Pond the slope of the curve for mother fish 33-37 mm. in length on May 19 was 4.5; for fish 35-39 mm. long it was 2.2; and for the fish above 37 mm. in length it was 1.0. Similarly diminishing slopes in the curves held for subsequent broods carried by the parent stock. Among the offspring in Parr's Pond the slopes of the curve for the first brood of young carried by the second brood of mature offspring gradually decreased from 5.3 for fish 24-28 mm. long, to 4.5 for 26-30 mm. fish, to 4.2 for those individuals longer than 30 mm. Again, similar curves held for data on the young carried by other offspring in that pond.

In the slow-growing fish from the Sanitary District Lake (Fig. 9) the reverse was true; that is, the rate of increase gradually increased with the length of the gravid female. In the first brood of young carried by the parent fish in that population the slope of the curve for the individuals 32-37 mm. long was 0.6. From that figure there was an increase to 3.4 for fish 35-39 mm. long, and to 5.1 for those fish of a greater length than 37 mm. Similar curves were obtained from the data for the 2 subsequent broods.

The reason for such diversity in the relationship between the size of the brood and the length of the mother fish is not readily discernible. Presumably some metabolic factor is involved. Physiological dif-

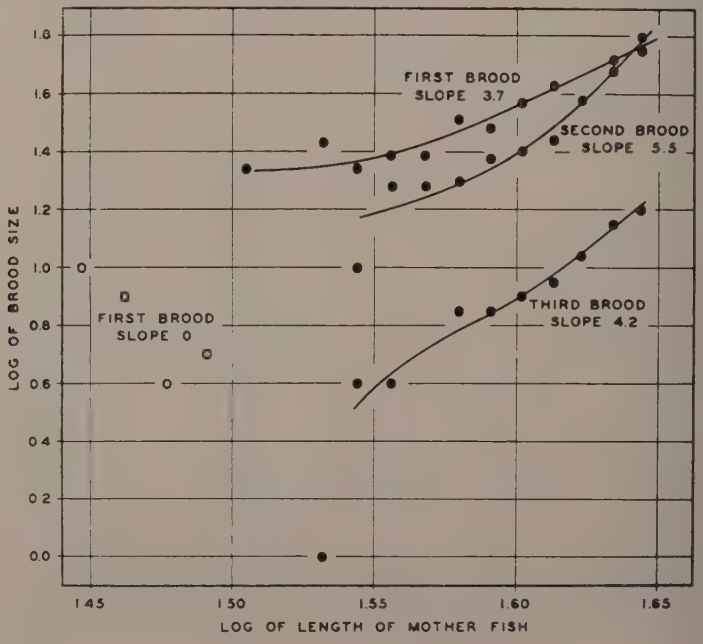

FIG. 9. The rate of increase in the size of the brood as correlated with the increase in the length of the mother fish in successive collections from the Sanitary District Lake, Cook County, Illinois, during 1939, as given in Table 12. The slope values represent the exponent of the rate of increase as indicated by a straight line drawn through the main part of each curve. The solid dots represent the parent females, the open circles the gravid offspring.

ferences, dependent to a certain extent on environmental conditions, may account for the increased or decreased rates of increase. That the space provided by the mother fish is not the sole determining factor for the number of young per brood is evident for several reasons: (1) the size of subsequent broods decreases with increasing size and age of the mother, (2) the approaching end of the reproductive period apparently has some marked effect on the fecundity, and (3) there is considerable difference in the fecundity of fishes of the same size from different localities.

Decrease in the Size of Broods with Increasing Age

With the single possible exception of the first brood born to the parent stock, females of the same category in a single population have fewer young per brood as the season progresses. This decrease in fecundity with increasing age holds for both parent fish and offspring (Tables 11-13; Figs. 7-10). The average number of young per brood for parent females of various lengths from the Argonne Woods Pond and the Sanitary District Lake show that even though the actual length of the females increased there was a marked decrease in the size of the broods in successive collections. Similarly, among the mature offspring in Parr's Pond the broods carried by females taken in the second collection were smaller than those taken in the first sample even though the average size of the gravid offspring increased nearly 3 mm. There was a further decrease in brood size as the summer progressed in all cases.

The comparisons given below of the mean numbers of young carried by parent females of similar lengths on June 9 and July 13 and on July 13 and August 14 for the Argonne Woods Pond and the Sanitary District Lake clearly show those differences:

Mean sizes of broods—Argonne Woods Pond

Date	Females 46-55 mm. June 9	Females 46-55 mm. July 13	Females 38-44 mm. July 13	Females 38-44 mm. Aug. 14
Number of females	63	194	179	3
Average size	49.3	52.2	52.5	52.3
Average number of embryos	210.4	152.9	153.3	42.7
Decrease		57.5		110.6

Mean sizes of broods—Sanitary District Lake

Date	Females 35-44 mm. June 9	Females 35-44 mm. July 13	Females 50-55 mm. July 13	Females 50-55 mm. Aug. 14
Number of females	67	236	228	449
Average size	38.3	40.3	40.4	40.6
Average number of embryos	30.7	28.4	28.8	8.8
Decrease		2.3		20.0

The young-of-the-year fish in Parr's Pond show agreement with the parent fish in the 2 ponds near Chicago in that the size of the broods decreased as the summer progressed:

Mean sizes of broods—Parr's Pond

Date	Females 27-45 mm. July 31	Females 27-45 mm. Aug. 14	Females 35-46 mm. Aug. 14	Females 35-46 mm. Sept. 19
Average number females	330	324	167	25
Average size	32.2	34.9	38.2	40.5
Average number of embryos	47.4	34.3	49.9	17.0
Decrease		13.1		32.9

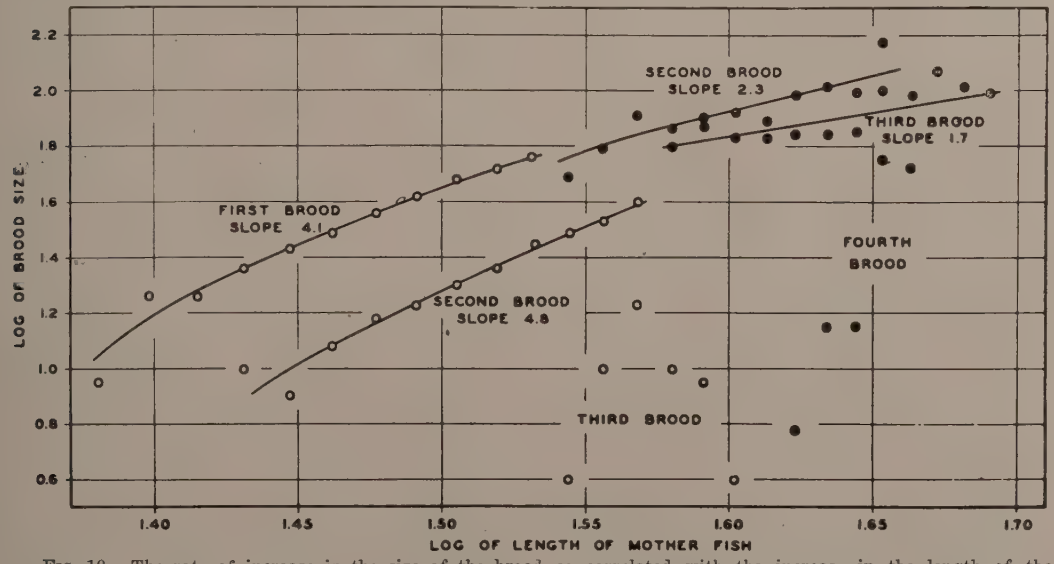

FIG. 10. The rate of increase in the size of the brood as correlated with the increase in the length of the mother fish in successive collections from Parr's Pond, Macon County, Illinois, during 1940, as given in Table 13. The slope values represent the exponents of the rate of increase as indicated by a straight line drawn through the main part of each curve. Here the solid dots represent the first brood of offspring that became gravid, the open circles the second brood.

Among the offspring from the Argonne Woods Pond the only comparable size in gravid fish was 41 mm. The 3 fish taken July 13 averaged 107 young per brood whereas the 4 individuals collected August 14 carried an average brood of only 16 embryos, a decrease of 91.

Further evidence of the decrease in productivity with increasing age is manifest by the decrease in the average number of young produced by fish of the same category in successive collections, despite the increase in the average size of the parent. In the Argonne Woods Pond (Table 11), the average size of the broods on June 9 was 205.4 for the parent females, which averaged 48.8 mm. in length. That average dropped to 155.7 young on July 13, when the parents averaged 52.6 mm., and the few parent fish remaining on August 14, averaging 52.3 mm., carried an average of only 42.7 embryos. Among the offspring from the Argonne Woods Pond the average number of young was 34.3 on July 13 and decreased to 15.6 on August 14, despite the fact that the average size of the mothers had increased from 35.3 to 42.2 mm. A similar perusal of the data from the Sanitary District Lake (Table 12) reveals that the average number of young carried by parent fish on June 9 was 30.4 and that it fell to 28.4 on July 13 and dropped still further to 8.8 on August 14, whereas the average size of the mothers on those dates was 38.8, 40.2, and 40.5 mm. In Parr's Pond (Table 13), where only gravid offspring were collected in numbers sufficient to warrant comparison, the average brood contained 44.5 embryos on July 31, 37.5 embryos on August 13 and only 17.1 embryos on September 19, although the respective lengths of the female were 31.6, 35.4, and 40.5 mm. Here it is obvious that, even though the mother fish actually increased considerably in length, the average size of the broods became markedly smaller as the fish grew older.

Many factors might have caused the observed decreases in the size of successive broods. Among the possible causes are: deterioration of natural conditions (water temperatures, chemical composition of the water, water level, availability of food, etc.); changes in population pressure; or some physiological change in the fish itself. An unfavorable change in the habitat seems unlikely, inasmuch as it would probably affect all fish alike, not only a selected group such as the gravid females. The effects of specific physical and chemical conditions on fecundity are not obvious. It may be that the size of successive broods decreased because of the liberation of large numbers of young early in the season with a consequent development of population pressure. This, however, seems unlikely as any compensation in the population due to increased numbers of individuals would probably take place after the young had been liberated and not in the reproductive mechanism. Physiological changes in the mothers appear much more likely to be the causative factors.

In the experiments referred to under the discussion of the length of the gestation period (see page 13) the numbers of young in each of the broods born to each segregated female were counted. The data (Table 14) again show that the broods become smaller as the female becomes older. The second broods of 3 of the 4 females were slightly larger than the first whereas in the other individual the second brood was smaller than the first. The third brood was consistently smaller than the second. The last brood in the 2 fish that bore 4 broods was the smallest. The decrease in the size of these broods was certainly not the result either of a deteriorated habitat or of population pressure. The aquaria were kept clean and the young were removed soon after birth. Apparently the decrease in the size of the broods with increasing age of the mother fish is a physiological phenomenon closely associated wtih the production of young.

TABLE 14. Sizes of successive broods born to female mosquitofish under laboratory conditions, 1940.

Fish Number	Number of Young			
	First brood	Second brood	Third brood	Fourth brood
1	24	28	7	..
2	34	36	12	6
3	42	47	18	8
4	21	16	3	..
Average	30.8	30.8	10.0	7.0

SENILITY

It is a well known fact that poeciliids, unlike many other fishes, have a definite period of sterile senility following reproduction. Evidence presented in the foregoing paragraphs shows that the period of reproduction in female mosquitofish, under natural conditions in Illinois, embraces only a single series of broods. Further, it has been pointed out that there is a marked decline in the reproductive efficiency of the female with increasing age; that is, the size of the broods becomes smaller as the reproductive period progresses.

The number of broods in the series cast by any individual depends on the age of that individual at first maturity and on the time of year at which reproduction began. A female becoming gravid during her first summer of life (age, 1 month) would bear, on the average, 2 broods of young, whereas an individual that did not begin to reproduce until the second summer of life (age, 8-10 months) would liberate approximately 4 broods. Further, the numbers of young per brood liberated by the females that reached maturity when only a month or so old were considerably smaller than those released by individuals that did not become gravid until the second summer of life.

The females that reached maturity during their second summer were obviously better suited physiologically to bear young than were those which reached maturity at an early age. The offspring in the

Argonne Woods Pond on July 13 were carrying fewer eyed embryos per brood on the average than were parent fish of the same lengths on May 19.

Senility among mosquitofish is not a function of age alone. Females that began to bear young when only a month or so old, apparently were physiologically more senile at an age of 4 months than were those which did not begin to reproduce until they were in their second summer of life. The older fish enjoyed a considerably longer and more fruitful period of reproduction than did those which reached maturity at an early age.

Further evidence of senility in female mosquitofish was found in the collections made in 1938. Among the fish taken in Pond 2 (Table 2), all individuals 33 mm. or more in length, with the single exception of the largest female (53 mm. long), were gravid. That non-gravid female was one of the parent stock (1 annulus on the scales) that apparently had completed her reproductive period. A microscopic examination of the ovary revealed no ova and the over-all aspect of the organ suggested that the fish was in a senile condition. Another female in similar condition was taken from the Argonne Woods Pond, August 14, 1939. It was a parent female 50 mm. long, and was not gravid, whereas all other females of comparable size or larger were carrying young (Table 9).

Variation in the Size of Broods According to Locality

The first indication of the relationship between the number of young per brood and the locality in which the mother fish lived was evident in the collections taken from 5 ponds near Chicago during August 1938 (Table 10, Fig. 7). Size for size the females in Pond 1 carried larger broods than did those from any of the other ponds. However, the largest brood, 174 eyed embryos, was taken from a female 54 mm. long from Pond 5. That fish and another female 57 mm. long from the same pond were known to be members of the parent stock (see page 7). It is likely that all other gravid females in the collection were young of the year. The reason for so great a diversity in the fecundity of the females in Ponds 1-5 is not obvious. The ponds were in the same general region and from all outward appearances were similar. It is unlikely that there could be any genetic cause inasmuch as the fish in all of the ponds came from the same stock and the plantings were made about the same time. Perhaps the most acceptable explanation is in the probability that the individual fertility of the ponds may have differed considerably.

More conclusive evidence that the locality or environment influences the fecundity of the female mosquitofish is shown by the data collected from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond (Tables 11-13; Figs. 8-10). On June 9, the parent female from the Argonne Woods Pond carried an average of 205.4 embryos per brood whereas, on the same day, fish of the same age-category from the Sanitary District Lake contained broods of only 30.4 eyed embryos. No collections were made from Parr's Pond until July 31. Although parent fish of the same length were seldom collected from the Argonne Woods Pond and the Sanitary District Lake on the same day, it is obvious that the fish in the former pond were much more prolific than those from the latter. Gravid parent fish 43 and 44 mm. long were taken from both ponds on June 9 and the mean size of the broods from the Argonne Woods Pond was 158 young as compared with 55.5 young from the Sanitary District Lake. The only parent female taken from Parr's Pond was 52 mm. long on July 31 and contained 154 eyed embryos. Fish of similar size were taken from the Argonne Woods Pond on July 13 and August 14 and carried broods of 155 and 30 young respectively on those 2 dates. Although such data are meager, it is evident that the parent fish in Parr's Pond were as prolific, if not more so, than those from the Argonne Woods Pond.

So far as the mature offspring in the 3 ponds are concerned, the fecundity of the fish in Parr's Pond was greater than that of either the Argonne Woods Pond or the Sanitary District Lake. The offspring in Parr's Pond not only liberated more broods of young (page 21), but the broods were larger than those from either of the 2 ponds near Chicago. Among the gravid offspring in Parr's Pond on July 31, the size of the broods ranged from 9 to 149 with an over-all average of 44.5 embryos, whereas in the Argonne Woods Pond on July 13 the number of embryos per brood among the offspring ranged from 8 to 107 with an average of 34.3 and in the Sanitary District Lake the offspring were not yet bearing young. On August 13 in Parr's Pond, the average number of young carried by offspring was 37.5 while on August 14 in the Argonne Woods Pond, the gravid offspring carried an average of 34.3 young and those in the Sanitary District Lake contained an average of only 5.3 young per brood. Furthermore, the offspring in Parr's Pond were still bearing young in October whereas no gravid fish were taken in the collections subsequent to August 14 in either of the 2 ponds near Chicago.

Comparison of brood sizes of offspring of similar lengths from these 3 ponds, where possible, clearly shows that the population of Parr's Pond was more prolific than either of the other two:

Mean sizes of broods from fish of similar size range:

Size range	Parr's Pond July 31	Argonne Woods Pond July 13
31-41 mm.	58.6	34.3
	Parr's Pond August 13	**Argonne Woods Pond August 14**
41-44 mm.	68.3	15.6
	Parr's Pond August 13	**Sanitary District Lake August 14**
28-31 mm.	13.1	5.3

The differences in fecundity among the fishes of the 3 ponds mentioned above are to be attributed to

the marked differences in the fertility of those bodies of water. Parr's Pond supported an abundant crop of plankton, probably because of the continuous deposition of excreta by livestock pastured in the area. At the time the Argonne Woods Pond and the Sanitary District Lake were selected as sites for collections during 1939, it was felt that the former pond, because of the heavy growth of various aquatic plants, was considerably more productive.

Among the early investigators it was believed that the broods were rather small. Ryder (1882) suggested that the maximum size of a brood in Gambusia probably did not exceed 25 or 30 embryos and elsewhere (1883) stated that the number may be as few as 15 or 20. Smith (1912) recorded broods ranging from 85 to 134 fully developed embryos from females 45 to 50 mm. long taken in June 1912 from the Lower Potomac River and other broods of 18 to 30 embryos from fish of the same size from the same river in August 1890. His observations led him to suggest the production of more than 1 brood per season and that the second brood was smaller than the first because the presence of the developing embryos of the first brood inhibited the development of an equally large number in the later brood. Kuntz (1913) noted that the larger females usually gave rise to larger broods of young than did the smaller ones. He reported an average of 33 embryos per brood from a limited number of fish 50 to 60 mm. long from Beaufort, N. C., during the summer of 1912. The largest brood was 76 embryos. The size of the brood of the smaller fish ranged from 2 or 3 to about 20 individuals. The relatively small size of the broods was attributed to the fact that the fish were collected during the late summer and had probably passed the peak of their reproductive period. Seale (1917), working with aquarium-reared fish, recorded a maximum brood of 49 embryos.

In his early work on the mosquitofish, Hildebrand (1917) reported a brood of 63 embryos from a single female. Later (1921) he stated that the number of young produced at one time varies greatly, ranging from a few to a couple of hundred and listed broods of 120, 163, and 211 young. However, he said that the average number of young probably does not exceed 40, and that the number varied with the size of the female. In aquarium experiments he showed that a single female liberated as many as 345 young, consisting of 2 generations, during a single season.

In a rather comprehensive study of the fecundity of *Gambusia a. affinis*, Barney & Anson (1921b) reported broods ranging up to 226 embryos in a series of 800 females collected during 4 summers near Mound, Louisiana. The largest brood, 226 young, was carried by a female 43 mm. long, standard length (ca. 51 mm. total length). The smallest brood was probably from one of 5 fish 23-24 mm. long, standard length (ca. 31 mm. total length), that had an average of 8.6 embryos per brood although the actual size of the smallest brood was not given. The smallest gravid female observed was 17 mm. long, standard length (ca. 22 mm. total length) and contained 9 embryos in August. Those workers combined the data collected from the various waters during the 4 summers into bi-monthly periods in an attempt to show the relationship between the fecundity and the length of the female. They noted

> ". . . that fecundity in this species increases markedly as the length of the female increases. From the average count of embryos based on April and May observations, the fecundity of females of 2.3 to 2.4 cm. length is doubled when the fish is about 3.2 cm. long; the fecundity is about quadrupled when the length 3.5 to 3.6 cm. is reached. With each 2 millimeters' growth thereafter the fecundity is greatly increased."

The measurements given by Barney & Anson were standard lengths. They noted further

> ". . . that in April and May the average number of embryos found in sixteen pregnant females was 108.1; for June and July, 60.3; for August and September, 40.5; and for October, November, December, January and February, 0; in fact, in these five months there were no embryos found in females of any size. . . ."

It is unfortunate that those workers failed to show the variation in size in successive broods from different populations and the possible differences in fecundity between the parent stock and the mature offspring.

De Buen & de Buen (1922), using fish that had been introduced into Spain for mosquito control, mentioned having found 38 advanced embryos in a female 46 mm. long. Artom (1924), who made his observations on fish transferred from Spain to Italy, counted broods of 15 and 34 embryos but did not indicate the sizes of the mother fish. Dulzetto (1928, 1934, 1935) in observing 78 parturitions among 29 mosquitofish living under conditions simulating those in nature, recorded broods that ranged from 4 to 97 embryos. The individual sizes of the mother fish were not given. Of the 29 individuals under observation by Dulzetto, 2 gave birth to only 1 brood, 9 bore only 2 broods, 14 bore 3 broods, and only 4 gave birth to 4 broods. The size of the first brood was usually smaller than the others and, in general, the second brood was the largest.

Sokolov (1936), working with Gambusia placed in rice fields in Central Asia as a mosquito-control measure, found that the fecundity depends on the size and age of the fish. He stated that mosquitofish are most prolific when they are 45-50 mm. in length which corresponds to an age of 2 or 3 years. Broods that ranged from 9 to 151 embryos were recorded but no mention was made of the individual lengths of the mother fish. Maksudov (1944) reported that there is a constant ratio between the weight of the mother fish and the weight of the offspring. He associated the increase in fecundity with the intensity of metabolism based on oxygen consumption. Prior to fertilization there is a greater oxygen demand because of the increased need for energy in the formation of yolk materia, etc., in the maturing ova. After fer-

tilization there is a sharp decrease in the demand for oxygen because the embryos use the food material stored in the mature egg.

The largest single brood of mosquitofish recorded in the literature was one of 354 young (Bonham, 1946). The fish carrying those embryos was taken from a small pond in Texas. Unfortunately, the size of the mother fish was not given.

Length Distribution and Growth Rate of Males

Respective totals of 3,061 and 4,129 male mosquitofish were taken in 6 collections from each of the Argonne Woods Pond and the Sanitary District Lake during 1939 and a total of 3,351 males were collected in 4 samples from Parr's Pond in 1940. The mature males were distinguished from the immature individuals by a microscopic examination of the anal fin; those fish in which the formation of the gonopodium was complete were considered as mature and all others as immature.

Mature males were present in the last 5 collections from the Argonne Woods Pond, whereas such fish

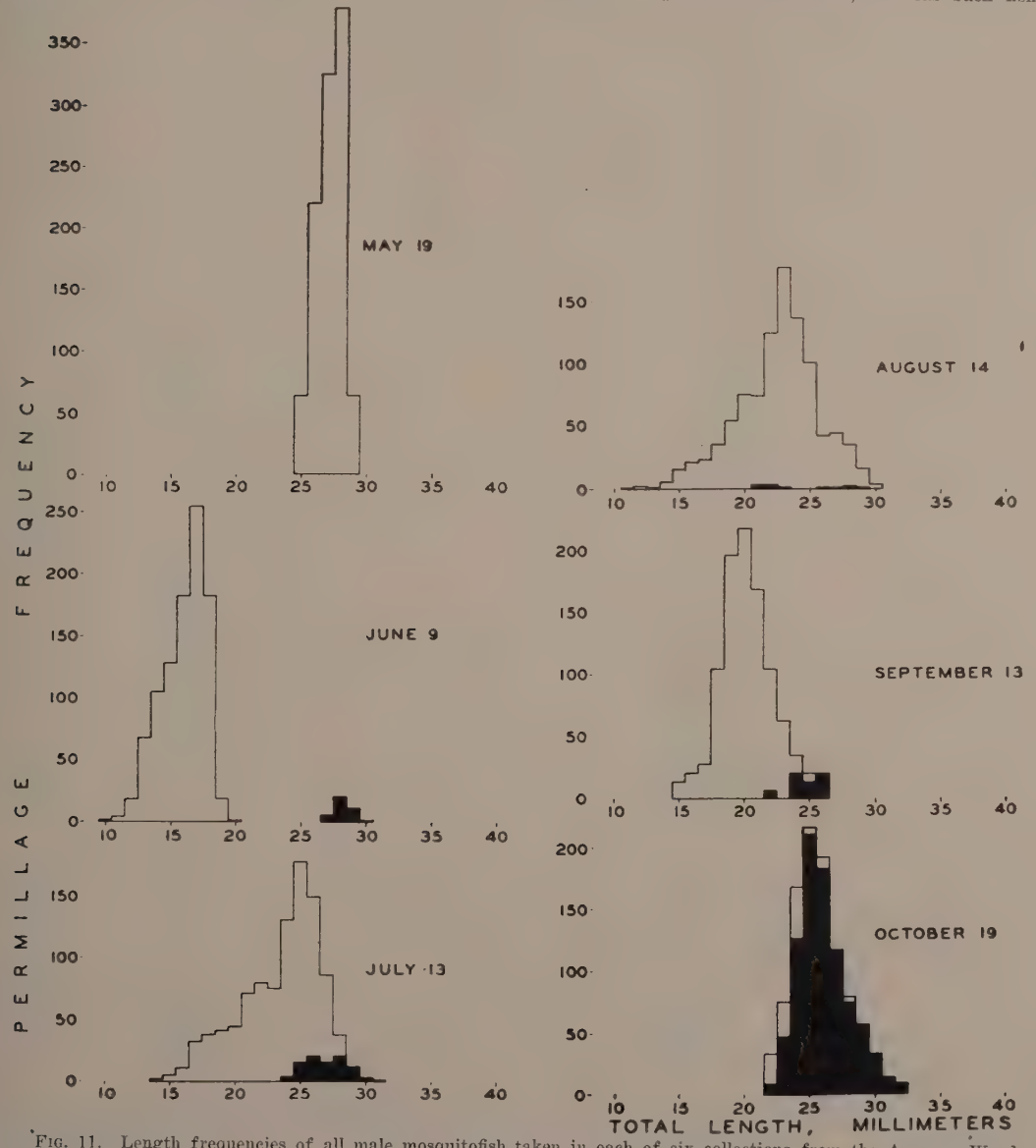

FIG. 11. Length frequencies of all male mosquitofish taken in each of six collections from the Argonne Woods Pond, Cook County, Illinois, during 1939. The shaded areas indicate the mature males, the unshaded the immature.

were present in all collections from the Sanitary District Lake and Parr's Pond. Immature males were present in all collections from the Argonne Woods Pond and Parr's Pond and were lacking only in the June 9 collection from the Sanitary District Lake. The size ranges and average lengths for all males in each collection are given in Table 15 for the Argonne Woods Pond, in Table 16 for the Sanitary District Lake and in Table 17 for Parr's Pond. These same data are graphically represented in Figures 11, 12, and 13 respectively.

Age and Growth of Mature Males

It has been shown by Turner (1941) that males of Gambusia, unlike the females which continue to grow until they die, stop growing, or grow only very little after the gonopodium has been completely formed.

The largest mature males taken in the collections

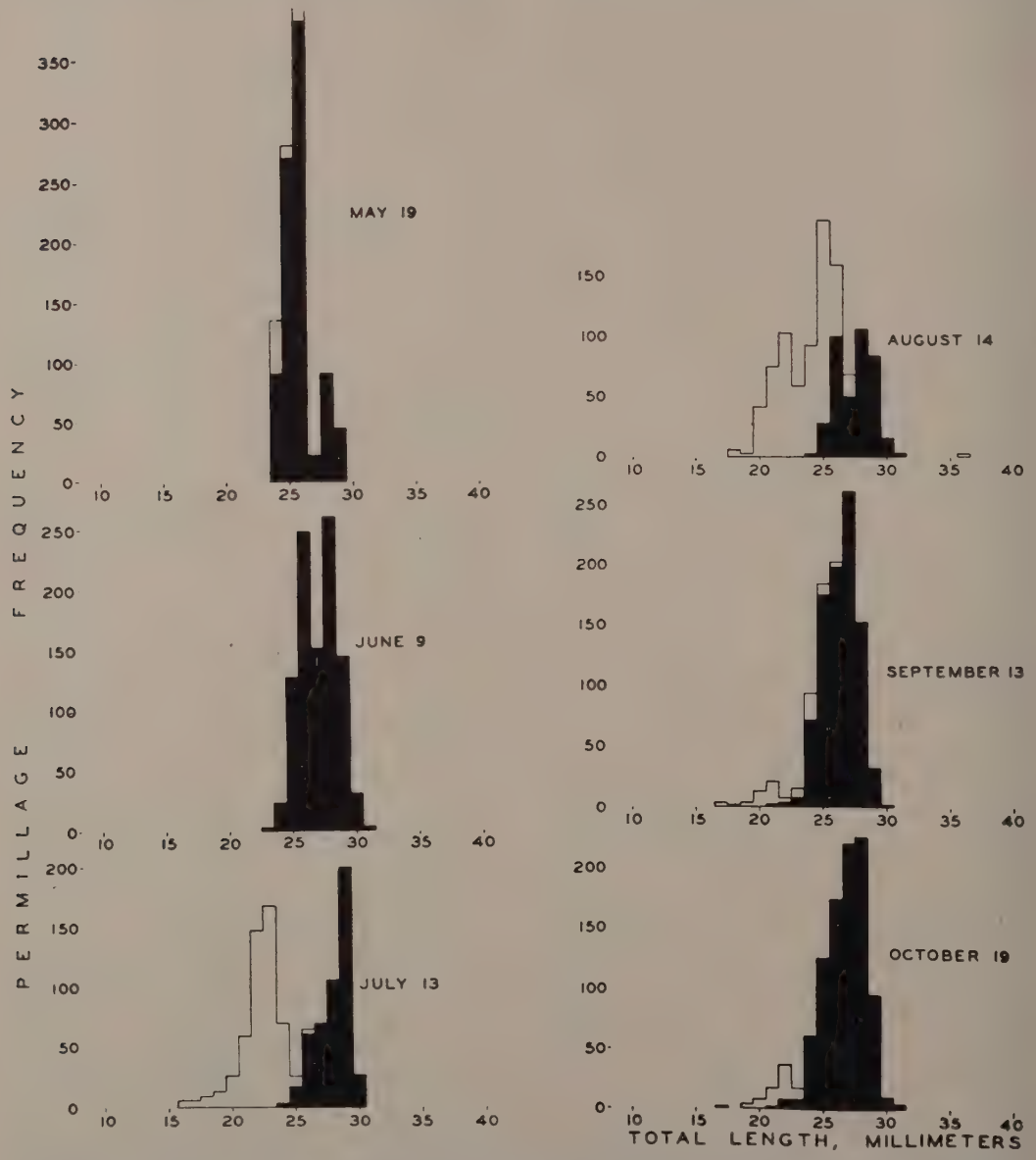

FIG. 12. Length frequencies of all male mosquitofish taken in each of six collections from the Sanitary District Lake, Cook County, Illinois, during 1939. The shaded areas indicate the mature males, the unshaded the immature.

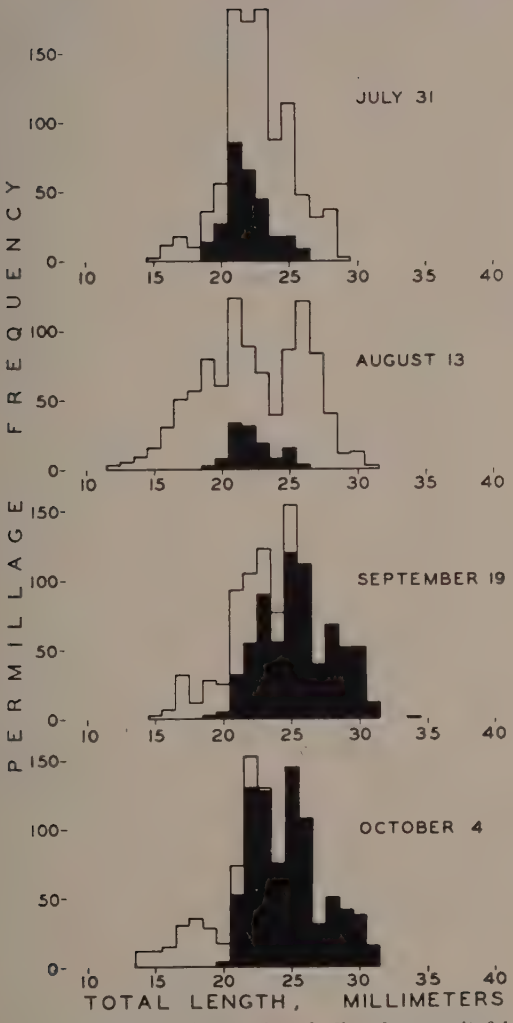

FIG. 13. Length frequencies of all male mosquitofish taken in each of four collections from Parr's Pond, Macon County, Illinois, during 1940. The shaded areas indicate the mature males, the unshaded the immature.

from the Argonne Woods Pond and Parr's Pond were 31 mm. long, whereas those from the Sanitary District Lake were 32 mm. in length. The largest mature male ever collected by the writer was 37 mm. long. That fish was taken from a small pond in Cook County, Illinois. The smallest mature males were present in the July 31 and August 13 collections from Parr's Pond (19 mm.).

Data from the May 19 collections from the Argonne Woods Pond and Sanitary District Lake show that some of the survivors from the preceding summer (1 annulus on the scales) were still immature. However, those fish had all reached maturity by June 9 and were disappearing from the population by the middle of August. None of the original planting of males in Parr's Pond were found in the collections.

TABLE 15. Numbers of mature and immature male mosquitofish showing minimum, average, and maximum total lengths in millimeters in each of six collections from the Argonne Woods Pond, Cook County, Illinois, 1939.

Date of Collection	Immature				Mature			
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
May 19	19	25	27.2	29	..	..	....	..
June 9	646	10	16.0	20	27	27	28.2	30
July 13	839	14	23.2	29	86	24	26.9	31
August 14	1,087	11	22.5	30	12	21	24.2	29
September 13	133	15	20.0	25	9	22	24.7	26
October 19	24	22	23.7	28	188	22	26.1	32

TABLE 16. Numbers of mature and immature male mosquitofish showing minimum, average, and maximum total lengths in millimeters in each of six collections from the Sanitary District Lake, Cook County, Illinois, 1939.

Date of Collection	Immature				Mature			
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
May 19	6	24	24.5	26	84	24	25.9	29
June 9	...	..	....	..	288	23	27.1	31
July 13	174	16	22.2	26	165	24	28.0	30
August 14	194	18	23.5	27	126	24	27.5	31
September 13	46	17	22.1	26	476	21	26.3	30
October 19	35	17	21.4	23	476	21	26.8	31

TABLE 17. Numbers of mature and immature male mosquitofish showing minimum, average, and maximum total lengths in millimeters in each of four collections from Parr's Pond, Macon County, Illinois, 1940.

Date of Collection	Immature				Mature			
	Number	Length			Number	Length		
		Min.	Av.	Max.		Min.	Av.	Max.
July 31	400	15	23.0	29	158	19	22.0	26
August 13	1,028	12	22.3	31	151	19	22.4	27
September 19	180	15	21.2	34	418	19	25.5	31
October 4	193	14	18.4	23	823	20	24.8	31

Young-of-the-year males of the first broods which matured by July or August also died before the end of the summer. Such mortality of young-of-the-year males is clearly indicated in the data of the fast-growing populations of Parr's Pond and the Argonne Woods Pond but is rather obscure in those of the slow-growing population of the Sanitary District Lake. Thus it appears that the life span of the male mosquitofish is no longer than that of the female.

SIZE AND GROWTH OF IMMATURE MALES

If the rate of growth is constant for all individuals throughout the summer, and the formation of the gonopodium is a function of age alone, all males

would reach maturity at approximately the same length. Obviously such is not the case as indicated by the extent of the size range in the mature males from all collections. Some individuals grow faster than others before reaching maturity or else they mature later after having grown for a longer period of time. Such variations in length of time before maturity is reached are probably due to environmental factors such as availability of food, temperature, etc., or may be due to the physiological makeup of the individual fish.

The length-frequency distributions for the males in the Argonne Woods Pond (Fig. 11) indicate that some of the individuals which were immature on June 9 had become mature by July 13. Those fish which reached maturity during the interim had grown approximately 10 mm. as reflected by the change in their length distribution from 10 to 20 mm. on June 9 to one of 24 to possibly 31 mm. on July 13. Obviously not all of the immature individuals of June 9 became mature by July 13 inasmuch as there were large numbers of fish which were apparently members of the first brood of offspring that were still immature on the latter date. Data from the Sanitary District Lake (Fig. 12) indicate similar conditions although the rate of growth was not so rapid. Those fish which were immature on July 13 evidently did not attain maturity until September and the length distribution showed an increase, from 16-26 mm. on July 13 to 21-31 mm. on September 13, or about 5 mm. In Parr's Pond, where even more rapid growth of the population than that in the Argonne Woods Pond took place, the length distributions of individual broods were marked by the presence of other broods.

Evidence from the length frequencies of the male offspring which mature during their first summer indicated that the fast-growing individuals reach maturity at a younger age than do those which grow more slowly. The average size of the mature males taken in all collections from Parr's Pond (24.5 mm.) was considerably smaller than that for the Argonne Woods Pond (26.4 mm.) which in turn was smaller than that for the Sanitary District Lake (26.8 mm.).

Although immature males 34 mm. long were taken on several occasions, none were collected which had a greater total length than that of the largest mature male (37 mm.).

Size and Age at First Maturity and Length of the Reproductive Period in Males

Inasmuch as male mosquitofish stop growing or grow only very little after the formation of the gonopodium is completed, the size of such fish at first maturity is closely approximated by the length at the time of capture. The considerable range in size of mature males of the same brood is explained by the fact that maturity is not reached by all individuals simultaneously. Turner (1941) found that some males became mature in 145 days (aquarium conditions) whereas others in the same group required 180 days.

The first mature male offspring were present in the same collection from the Argonne Woods Pond as the first gravid female offspring (July 13). All of these individuals were members of the first brood of young and consequently of the same age (6 weeks). The lengths of the mature male offspring ranged from 24 to 31 mm. (Table 15, Fig. 11) as compared with a length range of 31-41 mm. for the mature female offspring, all of which were carrying eyed embryos; a good indication that the individuals of both sexes had reached maturity as much as 2 weeks prior to the date of collection. The smallest mature males taken from the Argonne Woods Pond were 21 mm. long (August 14) and were probably members of the second brood of offspring. If that is the case, these smallest mature males were about the same age as those of the first brood when they reached maturity (4 weeks).

In the Sanitary District Lake mature male offspring were not collected until August 14 (Table 16, Fig. 12), whereas the first mature female offspring had been taken a month earlier (July 13). It appears that the mature males in the collection of August 14 might have been fish which survived from the preceding summer but examination of the scales showed that a few individuals were young of the year (no annulus). Most of the male offspring of this group, however, did not become mature until sometime before September 13 inasmuch as they were present as mature fish in the collection of that date. Here it is apparent that the males required a longer period of time to reach maturity than did the females. Perhaps this was due to a delayed development either because of a slower rate of growth, as exhibited by the rest of the population, or the lack of necessity to become mature when there was already an abundance of mature males present. The first mature male offspring were at least 8 weeks old by August 14 although they might have reached maturity at any time between that date and the date of the previous collection (July 13). However, the bulk of the group was immature on August 14 but had reached maturity by September 13 and so were somewhere between 8 and 13 weeks old when they became mature. The first mature male offspring collected from the Sanitary District Lake were not present in the samples until August 14, a month after similar fish had been taken from the Argonne Woods Pond. It is not meant to infer here that the fish in the Argonne Woods Pond reached maturity a month earlier than those of the Sanitary District Lake (the latter could have become mature at any time during the interval) but rather that the fish in a more slowly growing population required a greater length of time to reach maturity.

In Parr's Pond there was an abundance of mature male offspring when the first collection was made on July 31 (Table 17, Fig. 13). The identity of the brood or broods to which these individuals belonged is obscure as both parent fish and offspring were liberating young at the time (see page 19). In the

length distribution of the female offspring of the same collection, the modes indicate the presence of 2 broods (Table 8). The presence of very small mature males (19 mm. total length) together with a corresponding size range for such fish in each of the first 2 collections indicates that the males were reaching maturity at a rather constant size and rate. Also it is apparent that those individuals were reaching maturity at an early age as reflected by the length frequencies in successive collections. In such a rapidly growing population some of the immature males taken on July 31 had reached maturity by August 13 and some of those of the August 13 collection had become mature by September 19. However, it is clearly shown by those same length frequencies that all of the young-of-the-year males did not reach maturity at the same age. There were immature males present on July 31 larger than the largest mature males taken on August 13. It may be that (1) these larger fish had become mature and disappeared from the population during the interim, or (2) they had remained immature until later on, as fish of a comparable size were present in the collection of September 19. The latter hypothesis seems doubtful in the light of the findings from the Argonne Woods Pond and the Sanitary District Lake where males in the more rapidly growing population apparently reached maturity at an earlier age and died a short time after becoming mature.

Earlier workers have shown that in Gambusia one fertilization is sufficient for a female to develop and liberate a series of separate broods. Apparently the majority of the male offspring in the Argonne Woods Pond (Table 15, Fig. 11) reached maturity by October 19, but the absence of mature males in the collection of May 19 indicates that they did not survive to the latter date. Although no mature males were present in the May 19 sample, certainly such fish had been present before that time inasmuch as all of the females in that collection were gravid (Table 6, Fig. 2). It seems reasonable here to assume that some of the male offspring which had reached maturity the previous fall survived the winter and died after fertilizing the females early in the spring. Also it is obvious that some individuals remained immature over winter and attained maturity the following spring after the fertilization of the females had taken place. These late-maturing fish were not present in the samples after July 13. After that date young-of-the-year mature males served the function of fertilizing the female offspring as they became mature.

In the Argonne Woods Pond some of the immature males of the June 9 collection as indicated by the length frequencies (Fig. 11), had matured by July 13 but the numbers present as mature fish on the latter date were considerably fewer than the immature fish present a month earlier. The presence of large numbers of immature fish on July 13 indicates that many of the fish which were immature on June 9 may not yet have matured by July 13. However, the mode in the length distribution of the immature fish (25 mm.) on July 13 as compared with the mode on August 14 (23 mm.) indicates that the larger fish of July 13 had become mature and disappeared from the population within the space of a month. The mature males were considerably fewer than the immature individuals in all collections from the Argonne Woods Pond with the single exception of the sample taken in October. As shown previously, the males reached maturity at about the same or a little greater age than the females. Thus, if the mature males were as long-lived and had reached maturity at about the same age as the gravid females, they should have been present in as great or greater numbers than the immature males in the collections taken early in the summer. This, however, was not the case in the Argonne Woods Pond or either of the other 2 ponds. It was not until September or October, as the case in each pond may be, that the numbers of mature males equaled or exceeded that of the immature males of a size greater than that of the smallest mature male, whereas among the females, the number of gravid individuals were predominant throughout the reproductive season.

The evidence here presented indicates that males, although they reach maturity at about the same or a slightly greater age than the females, do not live so long after reaching maturity. This age after maturity among males varies according to the season at which the individual fish become mature. Those fish which mature in the fall may or may not live on as mature individuals until early the following spring, an interval of 7 to 8 months, whereas those of the first and possibly the second broods which mature during the same summer in which they were born probably do not live more than a few weeks after maturity has been reached. Furthermore, inasmuch as one fertilization is ample for a female to produce a complete series of broods, and the females die after one such period of reproduction, it seems reasonable to assume that males, after having contributed their bit to the reproductive process, should also disappear from the population as soon as the females have been fertilized.

There is no cycle in the reproductive process of male mosquitofish. Copulation takes place whenever the opportunity presents itself and mosquitofish are quite promiscuous in their breeding habits. Turner (1937) has pointed out that the production of sperm is practically continuous after the attainment of sexual maturity.

SEX RATIO AND DIFFERENTIAL DEATH RATE IN GAMBUSIA

The sex ratios for the mosquitofish, as reported in the literature during the first quarter of this century, indicated a disproportionate preponderance of females over males. Those ratios were perhaps inaccurate for two reasons: (1) the sole criterion used to distinguish sex was the presence or absence of the modified anal fin (gonopodium) and, (2) the gear used was very selective in that the seines or dipnets

were usually of such coarse mesh that only the relatively large specimens (mostly females) were captured. Also it is known, as will be discussed later, that there are considerable variations in the sex ratios in collections from the same body of water at different times of the year. In only a few instances has the writer found reference to a great preponderance of males in a collection.

Smith (1892) reported 68 females and one male in a single collection from the Lower Potomac River in Maryland and a ratio of 3 females to one male in collections from the Pasquotank River in North Carolina (Smith 1893). Fowler (1910) found males of Gambusia equally abundant with females in New Jersey waters. Hildebrand (1917) stated that the sex ratio in *G. a. holbrookii* in collections from Georgia was 8 or 9 females to one male. Barney & Anson (1921a) reported ratios of 5.8:1 and 4.5:1, females to males, in collections from Louisiana in 1918 and 1919 respectively. Those authors used the term "male" to indicate only fish in which the gonopodium was well developed; "young" fish were all those under 1.5 cm. standard length (ca. 21 mm. total length) with the exception of a few individuals which exhibited a modified anal fin; and "females" were all fishes more than 1.5 cm. standard length without any modification of the anal fin. This last group must have included many immature males. Hildebrand (1917) and Turner (1941) have called attention to the fact that the gonopod develops gradually, and at no definite age or length of the fish. In my 1939 collections, for example, some males were mature at 21 mm. and others were still immature at 30 mm.

Geiser (1924), in rearing 94 newly born mosquitofish (four complete and separate broods) to an age when the sex could be distinguished with certainty, was first to establish a 1:1 ratio (46 males, 48 females) between the sexes in this fish at birth.

Hildebrand (1927), in a study in which the sex of each individual was determined by a microscopic examination of the gonads, found the sex ratio to be 1:1 (1,295 females and 1,298 males) in "immature" mosquitofish less than 20 mm. long; a ratio of 1.48:1 (1,233 females and 712 males) in immature fish larger than 20 mm.; and a ratio of 4.4:1 (83,554 females and 19,596 males) in mature specimens. Those data were based on a large number of collections, taken in a great variety of waters representing nearly every environment in which Gambusia lives, over a period of five years (1921-1926). As late as 1927, however, Jordan stated that at birth the sex raio in Gambusia was 10 females to one male.

In four ponds from which de Buen (1930) made collections in 1928 and 1929, he found ratios of 253, 136, 153, and 388 females to 100 males. In all collections he used the presence of the gonopod as the criterion for distinguishing the sexes, and divided the fish into size groups similar to those of Barney & Anson (1921a). However, de Buen made no attempt to sex fish that were less than 20 mm. in length. Dulzetto (1934, 1935), in studying the sex ratio in 31 individual broods of Gambusia in 1934, found variations in the ratios among the broods from 37.5 to 400.0 females per 100 males but the sex ratio in all broods combined was very close to 1:1 (672 males, 661 females). During 1935, in a repetition of the 1934 study, Dulzetto found variations from 16.6 to 240.0 females per 100 males in 24 broods and again there was an over-all average of 1:1 (435 males, 443 females). The broods utilized in this study by Dulzetto were consecutive broods from 11 females in 1934 and 10 females in 1935.

Barnickol (1941) and Rice (1941) observed identical ratios of 7 females to one male in 1939 and 1940 respectively in mosquitofish taken from Reelfoot Lake, Tennessee.

There were considerable differences in the sex ratios in the 5 preliminary collections made by the writer near Chicago in August 1938. In those collections, females outnumbered the males in 3 samples whereas males were present in larger numbers in the other two. The length frequencies and numbers of individuals of each sex for each of the 5 collections, indicating a range of 26 to 652 females per 100 males, have been given in Table 2. In the combined samples the ratio was 190 females to 100 males (743 females, 391 males). The wide variation in these sex ratios might have been due to the small numbers of specimens in the collections. Segregation of the sexes which caused the individuals of one sex to be captured more easily than those of the other, also may have played a role in these and other collections discussed later.

In an effort to obtain further information regarding sex ratio in Gambusia, the entire collections of 1939 and 1940 were sexed. Furthermore, in 1940, newborn individuals of complete broods were separated from each other and allowed to reach maturity in isolation.

During the entire study a total of 19,150 female and 10,943 male mosquitofish were sexed. This shows an over-all sex ratio of 175 females to 100 males in a total of 21 collections taken during 3 summers (1938-1940). The numbers of topminnows of both sexes and the sex ratio (in parentheses) of the fish in each collection taken from the three ponds in Illinois during the summer of 1939 and 1940 are given in Table 18. From those data it is evident that the sex ratios differ considerably among individual collections from the same pond at different times during a single summer. The greatest difference in sex ratio was exhibited in the collections from the Sanitary District Lake during 1939. In those collections the ratios ranged from 2 to 537 females per 100 males, an extreme variation of 535. In the samples from the other two ponds the differences in sex ratio were not nearly so great—170 to 296 females per 100 males in Parr's Pond during the late summer of 1940. There appears to be an irregular increase in the frequency of females during middle and late summer. Such a shift might be explained by a differential death rate in the two sexes.

TABLE 18. Total numbers of male and female mosquitofish collected from ponds in Illinois, 1939-1940. The figures in parentheses indicate the sex ratio.

SANITARY DISTRICT LAKE COOK COUNTY, ILLINOIS			ARGONNE WOODS POND COOK COUNTY, ILLINOIS			PARR'S POND MACON COUNTY, ILLINOIS		
Collection date	♂♂	♀♀	Collection date	♂♂	♀♀	Collection date	♂♂	♀♀
May 19, 1939	89	2 (100:2)	May 19, 1939	19	39 (100:205)			
June 9, 1939	284	71 (100:25)	June 9, 1939	670	1,395 (100:208)			
July 13, 1939	328	1,538 (100:469)	July 13, 1939	926	1,577 (100:170)	July 31, 1940	553	494 (100:89)
August 14, 1939	322	1,728 (100:537)	August 14, 1939	1,109	2,218 (100:200)	August 13, 1940	1,172	1,042 (100:89)
September 13, 1939	1,541	2,244 (100:146)	September 13, 1939	144	373 (100:259)	September 19, 1940	601	920 (100:153)
October 19, 1939	1,439	2,973 (100:207)	October 19, 1939	211	624 (100:296)	October 4, 1940	1,036	1,144 (100:110)
Total	4,003	8,556 (100:214)		3,079	6,226 (100:202)		3,362	3,600 (100:107)

The sex ratios of the combined collections from each of the 3 ponds indicate that the relative abundance of the females is greater in standing populations than in newly populated ponds. The combined collections from each of the 2 ponds which supported well-established populations were roughly 200 females to 100 males, whereas in Parr's Pond the sex ratio in the combined collections was very nearly 1:1.

Laboratory experiments performed by the writer at the University of Illinois showed that the sex ratio of Gambusia at birth approximates 1:1. Newly born young of 4 entire broods (broods 1, 2, 3, and 4 in Table 19) were separated from each other and kept isolated, after the method of Geiser (1924), until maturity was reached. Also each of 2 entire broods (broods 5 and 6 in Table 19) of new-born young were placed in aquaria and allowed to grow to maturity in the presence of the other individuals. When the fish were thought to have reached maturity, the anal fin of each individual was inspected under the microscope for determination of sex. The date of birth, the date on which the fish were sexed under the microscope, the number of males and females and the sex ratio of each of the 6 different broods are shown in Table 19. Those data indicate a 1:1 sex ratio for *G. a. affinis* at birth.

If the sex ratio is 1:1 at birth, the preponderance of females later in life must be attributed to the differential death rate of the sexes. The literature on this subject is not extensive. Barney & Anson (1921a) found, in shipping several cans of Gambusia on a railroad journey of about 24 hours during August, that a large number of females but none of the males died. From this observation they stated that it was evident that male mosquitofish are somewhat more resistant to higher temperatures than are the females.

Geiser (1921, 1924), however, showed conclusively that the death rate for males of Gambusia in transit was greater than that of the females regardless of whether the temperature was high or low. In shipments made during cold weather (November) the death rate of males was about one and a half times that of the females, whereas in shipments made during warm weather (March) the males died at approximately two and a half times the death rate for the females. This greater death rate occurred in the males even though many of the females were heavy with young in March. Furthermore, Geiser (1924) indicated that female mosquitofish are more resistant to the rigors of winter than are the males. Of 100 fish, fairly equally divided as to sex, that were left outdors over winter, there were 32 survivors the following spring, of which only one was a male.

Hildebrand (1927) stated that, in a total of 41,073 adult fish, consisting of 7,357 males and 33,736 females, used in a series of experiments, there was a mortality of 29.75 per cent among the males and only 22.33 per cent among the females. These experiments include the study of the effects of high temperatures,

TABLE 19. Number of male and female mosquitofish indicating sex ratio in broods raised from birth (1940). The fish from broods 1-4 were segregated and the fish from each of broods 5 and 6 were raised together.

Brood number	Date of examination	Number of males	Number of females	Date of birth	Sex ratio (100♂:x♀)
1	IX:15	13	12	VII:30	100:92
2	IX:22	15	16	VIII:12	100:107
3	XI:28	10	11	IX:17	100:110
4	XI:28	15	14	X:19	100:93
Total or average		53	53		100:100
5	XI:28	19	20	VIII:4	100:105
6	XI:28	13	11	IX:6	100:85
Total or average		32	31		100:97
Total or average		85	84		100:99

of overcrowding the fish, and of using varying amounts of water in which to hold the fish. Hildebrand admitted that in some individual lots proportionately fewer deaths occurred among the males than among the females but stated that generally the reverse was true.

Although no special experiments on the resistance of Gambusia to adverse conditions were performed, many observations were made while transporting many thousands of those fish in Illinois and Michigan. It was noticed that mosquitofish were easier to transport over long distances than were most fish native to the area. In July 1944, 165 Gambusia of various sizes were placed in a 10-gallon milk can, approximately half full of water, in the trunk of the writer's automobile. These fish were the remnant of a group being planted for mosquito control. They remained in the can for a period of 9 days during which time they travelled a total of 782 miles. The water was not aerated nor were the fish given any food. Air temperatures reached into the nineties and certainly the temperatures in the car trunk might have exceeded 100 since no attempt was made to keep the car in the shade. Every other day the fish were observed to find out how they were getting on. At the end of 9 days the fish were removed from the milk can and in that period only 3 fish (all adult males) had died. The remaining 162 fish were sexed and if they were obviously males or females were liberated in a pond near Ann Arbor. Among the 162 fish there were 46 in which the sex was not readily recognizable. These were preserved and the sex determined under a microscope. The sex ratio of the entire lot was 166 females (103 fish) to 100 males (62 fish, of which 3 died during the experiment).

The experience of the writer has shown that in the great majority of cases male mosquitofish are much less resistant than are the females. On October 4, 1940, after a sample of mosquitofish from Parr's Pond was preserved, 7,750 fish were placed in four 10-gallon milk cans and taken to the North Pollywogs near Danville, Illinois, a distance of about 90 miles. The water in which the fish were carried was not aerated. At the end of the journey, the live fish were planted out and the dead ones were preserved. A total of 897 fish perished en route and of these 634 (70.7 per cent) were males and the remainder were females. Inasmuch as the sex ratio of the preserved collection was 110 females to 100 males it may be assumed that the ratio of the transported fish was similar.

In July 1944, Mr. Aden C. Bauman of the Missouri Conservation Commission took 149 Gambusia, from stock which had survived 3 winters in Michigan, to Columbia, Missouri. Those fish, some of which were gravid females, were placed in about 4 gallons of water in a can in the trunk of Mr. Bauman's car. The following is an excerpt from Mr. Bauman's letter of February 12, 1945: ". . . Returning to Columbia after a 2-day stop in Illinois, I had 137 live Gambusia. I kept them iced while in Illinois, at which time they were kept in a cellar. Twelve Gambusia, all adult males, died in transit. . . ."

It has been pointed out earlier that male mosquitofish, although they become mature at about the same age as the females, do not have so long a reproductive period. In the populations studied here the mature females were apparently longer lived than the mature males of the same brood. The females lived on to liberate several broods of young whereas the males, after fertilizing the females, disappeared.

In the considered opinion of the writer, Gambusia is easily transported from one place to another and, although both sexes are highly resistant to adverse conditions, the females, even though heavily gravid, are more resistant than are the males. The mature males, having a higher death rate than the females, are less numerous in long standing populations with the result that unequal sex ratios occur in adult stocks.

ACCLIMATIZATION OF GAMBUSIA TO NORTHERN ILLINOIS AND MICHIGAN

The natural range of the western mosquitofish has already been discussed (see page 5). Krumholz (1944) gave a short summary of the acclimatization of that poeciliid into northern Illinois and southern Michigan.

The first introduction of Gambusia into northern Illinois took place in 1923 when some fish, taken from a small pond on the campus of the Southern Illinois State Normal University at Carbondale, were transferred into a garden pool on the estate of Mr. Roland D. Whitman in Winnetka. That planting was augmented by another in 1925. In their new environment, near Chicago, the fish were subjected to much more rigorous climatic conditions than those to which they had been accustomed at Carbondale (Holcomb, 1941). However, Jordan (1927) stated that the director of the Illinois pond at Carbondale reported that a foot and a half of ice over the pond surface did not kill the mosquitofish as they were all hibernating in the bottom of the pond. Winters of this severity, although infrequent in the region of Carbondale, apparently aided the indigenous population of Gambusia in becoming hardier than other members of the same species farther south.

Evidently a considerable amount of good fortune accompanied the success of the plantings in Winnetka in 1923 and 1925. Clarke (1939), among the first to suggest the use of fish for mosquito control in the Chicago Region, stocked ponds on seven golf courses with mosquitofish from Carbondale in 1928. None of these fish survived the subsequent winter, so another attempt to introduce the fish from southern Illinois was made in 1929. Again the plantings failed, and in 1933 Clarke drew upon the stock in Winnetka for the first time and introduced the fish into 7 ponds in suburban Chicago in the hope of establishing hatcheries as a source for widespread stocking in later years. The method of dispersal proved satisfactory

and since that time more than 100 waters of various types in Cook County, Illinois, have been stocked with these "native" mosquitofish.

Plantings of southern Gambusia failed in the vicinity of Camden, New Jersey, and Philadelphia as will be shown later (page 35). Herms and Gray (1940) stated that Gambusia had been successfully introduced into the Puget Sound area, but the winters there, although farther north, are much more temperate than in the region of Chicago.

The western mosquitofish was first introduced into Michigan on June 30, 1941, when, through the courtesy of the Michigan Institute for Fisheries Research, several thousand specimens were transferred from the Argonne Woods Pond, Cook County, Illinois. These fish were taken to the State Fish Hatchery at Drayton Plains, Michigan, and during the course of the summer were introduced into a number of natural and artificial bodies of water. It seems unnecessary to describe each of these ponds other than to mention that 3 of the natural lakes were dystrophic, one was an acid bog, and one an eutrophic lake. The artificial ponds ranged in age from a few years to two decades and were all mud-bottomed with good growths of aquatic plants. All but 2 of the ponds were inhabited by other species of fish. Goldfish were introduced into one of the ponds sometime during the late fall of 1941 and in October 1944, 25 largemouth black bass fingerlings were placed in the pond.

The topminnows have successfully passed 5 winters in 2 of the artificial ponds. The other artificial ponds were not permanent. It is not known whether the plantings in the natural waters were successful although no topminnows have been collected from any of them.

Since 1941, mosquitofish have been introduced into natural waters in Michigan as far north as the Straits of Mackinac. Considerable numbers of Gambusia have escaped into the waters of the Clinton and Huron River systems from ponds which empty into those waters. The escaped fish may eventually establish populations in Lake St. Clair and the Lake Erie marshes. No adequate surveys of these lakes or streams have been made since the introductions of the mosquitofish and it is not known whether the fish have become established.

In 1942, some of the stock which had survived the preceding winter near Ann Arbor were sent to Dr. C. M. Breder, Jr., then Director of the New York Aquarium, in an attempt to get the species established in the region around New York City.

The fish which Mr. Bauman took to Missouri in July 1944 (page 32) were placed in three small shallow ponds in the vicinity of Columbia. An excerpt from Mr. Bauman's letter of Feruary 14, 1945, follows: "By freezing weather the Gambusia had reproduced until there were 'swarms' of them in all ponds. The freeze-up came December 1, 1944, and was followed by a 10-inch snow on December 10. By January the ice was 8-10 inches thick. On February 12, the ice had left our ponds and streams and we can say (with reservations) that spring is here. Also I can report that the hardy Gambusia from Michigan came through one of our hardest winters in years, whereas our river-bottom Gambusia always died when brought into this vicinity."

The minimum requirements necessary for the mosquitofish to pass a winter successfully in northern Illinois or southern Michigan, where there is a more or less continuous ice cover on the ponds during the winter months, are not easily defined. Where introduction of this fish into northern waters are contemplated, the first requirement is the selection of a brood stock from a region where climatic conditions simulate those of the new habitat as closely as possible. The brood stock should be obtained from the northern limit of the natural range of Gambusia or at least from a point where there is an ice cover on the ponds at some time during the year. The fish which were introduced into and became established in the Chicago region, were brought from Carbondale, Illinois. Although these fish did not come from the extreme northern limit of their natural range, freezing temperatures and ice formation occur during the winters and it may be assumed the fish were much more resistant to cold than fish brought in from a southern state. Perhaps the introductions in the vicinity of Philadelphia might have met with greater success if the introduced stock had come from the Chesapeake Bay area rather than from North Carolina.

The next problem is the selection of the proper type of pond in which to place the fish that it is desired to carry over winter. It has been the experience of the writer that the fish have a better chance of survival if they are introduced into a soft-bottomed pond, having a maximum depth of at least five feet, and free from other fish. The fish should preferably be placed in their new environment early in the summer so that the population can become well established before the ensuing winter. In some instances Gambusia will survive the winter in ponds of a lesser depth than five feet. The mosquitofish which had been left in Sam Parr's Pond, Macon County, Illinois, in the fall of 1940, survived the subsequent winter in water which had a maximum depth of 10 inches including an ice cover of about 4 inches during part of the winter.

In making the introductions of this exotic species into Michigan, ponds of different types, such as natural lakes with standing fish populations, bog ponds which harbored a few species of small fishes, impoundments with and without other fishes present, and hatchery ponds, were selected. Collections made the following spring showed the mosquitofish survived in all but one of the artificial bodies of water. One of the impoundments, a spring pond, contained brook trout *(Salvelinus f. fontinalis)* which had spawned successfully in the outlet stream. The floor of that pond supported a dense growth of Chara and the mosquitofish established themselves and successfully passed 3 winters in the pond. It is not known

whether the population is still present. In another impoundment there was a large population of sticklebacks *(Eucalia inconstans)* and the topminnows have become well established there. In still another pond, formed by excavating an intermittent marsh, Gambusia have withstood competition from large numbers of goldfish *(Carassius auratus)* and the population has increased so greatly that it has become a source of supply for further plantings since 1941.

The hatchery ponds, although often contaminated with various species of fish as yellow perch *(Perca flavescens)*, black crappies *(Pomoxis nigro-maculatus)*, pumpkinseeds, sticklebacks, minnows *(Notropis heterodon* and *N. heterolepis)* and other species, have supported Gambusia through 3 winters and have furnished a good source of supply for further introductions. Although these hatchery ponds have maximum depths of only 3 or 4 feet, there is a continuous flow of water through them during the winter which prevents the accumulation of harmful products of decomposition.

Gambusia have survived winters under the ice in northern Illinois in ponds having hydrogen-ion concentrations (pH) which ranged from 6.6 to 7.8 and total hardness (methyl orange alkalinity) readings between 12.8 p.p.m. and 320.4 p.p.m Also mosquitofish have failed to survive the winter in ponds more than five feet deep and whose pH and total hardness were well within the above range. Thus, although the range of tolerance to pH and total hardness of water shown by Gambusia is fairly wide, there are undoubtedly other factors which directly affect their ability to maintain a population.

In placing Gambusia in ponds where larger predaceous fish are present it is imperative that there is sufficient shallow water and aquatic vegetation to furnish ample cover.

Mosquitofish are capable of maintaining population in salt, brackish, or fresh water. Along the Gulf Coast they are found in all three of these habitats. Sicault (1934), in studies on the ability of *G. a. holbrooki* to become adapted to salt water in Morocco, found that it was possible to transplant the fish from fresh water into water, to which 12 grams of sodium chloride per liter had been added, without any apparent diminution of their larvivorous capacity. Also he found that by progressive adaption it was possible to transfer the topminnows from fresh water into water which approximated sea water in salt content (33 grams, sodium chloride per liter). In concentrations greater than 35 grams of sodium chloride per liter the Gambusia died.

GAMBUSIA AS AN AGENT FOR MOSQUITO CONTROL

That fishes are of value in mosquito control has been recognized since the latter part of the nineteenth century. An article which appeared in Insect Life (1891, 4:223) told of an English gentleman, who, having discovered that the mosquitoes which troubled him bred in large tanks kept for the purpose of storing fresh water, placed a pair of carp in each tank and succeeded in eliminating the insect pest.

The renowned entomologist, Leland Ossian Howard (1901), after obtaining information on the feeding habits and larvivorous capacity of Gambusia from Mr. W. P. Seal, Dr. H. F. Moore, and Dr. H. A. Veazie, was first to advocate the use of that poeciliid for the control of mosquito larvae. At that time, however, Seal, seemed inclined to believe that the pumpkinseed, *Lepomis gibbosus* (L.), a small sunfish, would be more satisfactory because its spiny rays would make it less likely to be eaten by larger fishes. Smith (1904) stated that no fish native to New Jersey could control mosquito larvae as efficiently as the mosquitofish and requested Seal to introduce Gambusia into that state. Accordingly, Seal (1910) transported 10,000 Gambusia and Heterandria from North Carolina to New Jersey and liberated them in the vicinity of Camden in 1905 (Howard 1910). This was the first attempt to introduce Gambusia into northern waters as a mosquito-control measure.

At the request of Mr. D. L. Van Dine, entomologist of the Hawaii Agriculture Experiment Station, Jordan sent Seale (1905) to Texas where the latter obtained fishes of 3 genera (*Fundulus grandis, Gambusia affiis,* and *Mollienesia latipinna*) in order to ascertain their effectiveness in the control of mosquito larvae. Gambusia proved to be the most efficient, but Seale took about 150 of each of the 3 kinds to Honolulu where they were again tested with similar results. That was the first time Gambusia was taken outside the continental United States for use in mosquito control. In 1913 Seale (1917) carried 24 mosquitofish in a glass jar in his stateroom from Hawaii to the Philippine Islands. In both island groups the topminnows have thrived and reproduced in large numbers.

Jordan (1926, 1927) was highly instrumental in having mosquitofish introduced into other countries. The American Red Cross and the International Health Board of the Rockefeller Foundation undertook a major portion of the responsibility of having such shipments made.

The introduction of Gambusia into Europe was due also to the efforts of Dr. Massimo Sella who, with the aid of the Red Cross, arranged to have shipments of *G. a. holbrookii* sent from Augusta, Georgia, to Spain and Italy in 1921. The lot sent to Spain arrived in good condition and the fish were placed in natural waters at Talayuela, Cáceres (de Buen and de Buen 1922), and thence transferred to other parts of the Iberian Peninsula. The fish intended for Italy failed to survive the trip but the following summer Sella (1926) took some of the stock from Spain to Italy. Because the mosquitofish from this original introduction were unable to withstand the winters in northern Italy, a shipment of *G. a. affinis* was sent from Carbondale, Illinois, to Trieste in November 1927 (Rivista die Malariologia, 6:999-1000).

Both of these subspecies of Gambusia have been widely distributed in Europe, Asia Minor, Africa,

and nearby islands. Because of the obscurity of some statements in the literature regarding the introduction of mosquitofish it has been impossible to separate the introduction according to subspecies. A list of places where *Gambusia affinis* is known to have been introduced in the Old World follows:

Albania	Greece	Romania
Algiers	Hungary	Russia
Austria	Italy	Sardinia
Bulgaria	Jugoslavia	Sicily
Caucasus	Macedonia	Spain
Corsica	Madagascar	Syria
Dalmatia	Mauritius	Tripolitania
Egypt	Morocco	Turkestan
Fezzan	North Caucasus	Turkey
France	Palestine	Ukraine
Georgia	Portugal	Union of
Germany	Reunion	South Africa
	Rhodes	

On the other side of the world, the mosquitofish has been distributed over a much greater area. Following its establishment in the Hawaiian and Philippine Islands, Gambusia has been introduced into the following:

Alaska	Cook Islands	Japan
Bonin Islands	Federated Malay	Marianas Islands
Borneo	States	New Ireland
Burma	Fiji Islands	New Guinea
Caroline Islands	Formosa	New Zealand
Celebes	Guadalcanal	Samoa
Ceylon	Hongkong	Siam
China	India	Tahiti

During World War II the United States Army has introduced mosquitofish into various island groups in the Pacific war theater not mentioned here.

In the Western Hemisphere the mosquitofish has been introduced into Ecuador and Argentina in South America; Porto Rico, the Virgin Islands and other of the Lesser Antilles; and into Arizona, California, northern Illinois, Kansas, Massachusetts, Michigan, Nevada, New Jersey, New Mexico, Ohio, Utah, Washington, Wisconsin, and probably other states.

Many of the introductions listed here have not been recorded in the literature and were graciously supplied by Mr. Alvin Seale, Professor Albert W. C. T. Herre, Professor Carl L. Hubbs, Mr. W. J. Phillipps, Mr. A. Cecil Harrison, and Mr. J. Lyell Clarke. Certainly there have been a great many instances in which this poeciliid was taken from one place to another for purposes of mosquito control of which no record was made, and consequently the lists given above are incomplete.

The natural range of *Gambusia affinis* has already been mentioned (page 10). Two other species, *G. nobilis* and *G. geyseri* are native to Texas. Still other members of the genus are native to the Gulf Drainage of Mexico and Central America, to both Coastal Drainages of Panama, and to the islands of Cuba, Hispaniola, Jamaica, and the Caymans and Bahamas.

The accompanying map (Fig. 14) shows the natural range of Gambusia and the localities into which *G. affinis* has been introduced throughout the world. Because of the large scale of miles, the areas of introduction as shown by the map are exaggerated in some instances. The facts at hand prompt the writer to agree with the late David Starr Jordan who stated that *Gambusia affinis* enjoys a wider distribution than any other freshwater fish.

The first, and most extensive, investigation on the use of fish in the control of mosquitoes undertaken by the United States Government was inaugurated as a public health measure in July 1915 (Smith 1916). The U. S. Bureau of Fisheries and the U. S. Bureau of Entomology, with the assistance of the U. S. Public Health Service, found it necessary to study the comparative efficiency of different fishes in controlling mosquito larvae and to ascertain how the effectiveness of the more suitable species was affected by the various conditions of depths of water, vegetation, and debris. An economic circular (Radcliffe 1915) was issued giving an account of the common fishes known to be of value in the control of mosquito larvae. The majority of this work was carried on in the "malaria belt" of the southeastern United States, and the mosquitofish was found to be more effective than any other species.

Hildebrand (1919, 1921, 1925), in an attempt to gain information relative to the actual value of Gambusia as eradicators of mosquito larvae and pupae under a wide range of conditions, made a thorough investigation over a period of four summers in the vicinity of Camp Hancock at Augusta, Georgia. The information secured from these experiments indicated that the mosquitofish had effected a reduction in the number of mosquito larvae and pupae amounting to 57.8 per cent in the anopheline and 80.8 per cent in the culicine populations. His data showed that reduction was materially greater in the number of pupae (74.2 per cent in anophelines and 85.1 per cent in culicines) than in the numbers of larvae (57.6 per cent in anophelines and 80.7 per cent in culicines). However, it was pointed out that the numbers of small larvae were reduced to a greater extent than were the large ones. In conclusion, Hildebrand listed the following reasons why *Gambusia affinis* is especially suited to antimosquito work:

> "(a) It seeks its food at the surface; (b) it is very prolific; (c) it gives birth to well-developed young, therefore requiring no special environment for depositing and hatching the eggs; (d) it lives and thrives under a large variety of conditions and frequents areas especially suitable for the support of mosquito larvae; (e) it usually lives and multiplies in ponds stocked with predacious fishes, providing it has very shallow water for refuge."

H. H. Howard (1920a, 1920b, 1922) obtained effective control over mosquito production by the use of Gambusia in a rural area of Hinds County, Mississippi. Howard's results from the use of fish as a control measure as compared with those achieved by oiling showed that: (1) fish control is less expensive—the cost is limited to the labor of capture and trans-

EQUATOR
NATIVE RANGE OF GAMBUSIA AFFINIS
NATIVE RANGE OF OTHER SPECIES OF THE GENUS GAMBUSIA
INTRODUCTIONS OF GAMBUSIA AFFINIS

FIG. 14. World map showing the native range of *Gambusia affinis* and other members of the genus and the different parts of the world into which the mosquitofish has been introduced as a mosquito-control measure.

portation; (2) fish control is less liable to accidents—the effectiveness of the oil film is quickly destroyed by winds, rains, and wave action, which have practically no effect on the efficacy of the mosquitofish as a control agent; and (3) fish control is more enduring in its results—one introduction of fish will suffice for a long period of time whereas oiling as a control measure is transient in its results and to be effective must be regularly and continuously practiced. In addition, Howard pointed out that the incidence of malaria during 1919 was only about 65 per cent of that of the preceding year.

Moore (1922), in an attempt to find a species indigenous to the North Atlantic States which would prove as valuable as Gambusia in mosquito control, made observations on the larvivorous capabilities of 29 species of fishes belonging to 9 families. He found 9 species which deserved special mention, and of these the pumpkinseed proved itself to be the best of the lot but not nearly so effective as the mosquitofish. Attempts were made to acclimatize Gambusia to the region of Philadelphia each of the three years of the investigation. All of the attempts failed, however, as had the earlier attempts of Seal, presumably because of the severity of the winters. Moore, realizing that annual reintroductions of mosquitofish into the many mosquito-breeding habitats in the region of Philadelphia would be impractical, even though such a practice would be of value, stated:

"... The planting of a permanent self-perpetuating stock only would suffice. Up to the present such stocking in the latitude of Philadelphia has failed. Just why Gambusia will not live over winter is not clear. The winter of 1920-21 was no more sever than those to which the top minnow is frequently exposed within its natural range, yet in four ponds in which they thrived during the preceding summer, not a single one appears to have survived. In an attempt to solve this second problem, it is proposed to search the northern border of the fish's range for a cold-resistant race or to attempt the isolation of such a race by selection and possibly by hybridization."

So far as is known to the writer, such a hardy race has not been established in the eastern United States.

Perhaps the most thorough treatise on the use of fish in controlling mosquitoes is the booklet "The Use of Fish for Mosquito Control" published by the International Health Board of the Rockefeller Foundation (1924). In addition to a discussion of the use of Gambusia in various parts of the United States, and also Spain and Italy, that paper includes a comprehensive treatment of the use of various kinds of fishes in all parts of the world where malaria is a menace. Recently, Gerberich (1946) annotated a bibliography of papers relating to the control of mosquitoes by the use of various kinds of fish.

Following the introduction of Gambusia into foreign lands, most workers in those countries have found that the fish are highly efficient in the control of mosquitoes. Near Rovigno, Italy, Sella (1929) found, in a single test pond where Gambusia was the sole agent for the control of anopheline production, that the fish were more effective during August than at any other time during the summer. The degree of control afforded by the mosquitofish for two successive years follows:

	1927	1928
May	2%	2%
June	16%	12%
July	70%	54%
August	87%	83%

Sella attributed the better control late in the summer to the tremendous increase in the population of Gambusia during June and July.

Conversely, Valli (1928) found that Gambusia were only 20 per cent effective in mosquito control. He attributed the poor control to the abundance of aquatic vegetation which contributed considerably to the hiding of the mosquito larvae.

In the region of Kavieng, New Ireland, Holland (1933) introduced *Gambusia affinis* into all known breeding places of Anopheles over a coastal area 72 miles long, containing 32 villages and 3,001 natives (960 children). No mosquitofish were placed in the remainder of the subdivision which included 35 villages and 3,438 natives (890 children). The spleen rate in the "treated" area fell from 24.6 in August 1930, to 4.2 in July 1932 whereas in the same time interval the spleen rate in the "untreated" part of the subdivision fell from 47.1 to 28.8. Moreover, in the treated area there was an excess of births over deaths for the first time on record.

Sokolov (1936), studying the value of Gambusia in mosquito control in rice fields in Turkestan, found that the extermination of Anopheles larvae by the mosquitofish amounted to about 90 per cent of the number of larvae in the control ponds. The results described by Sokolov clearly indicate the role of Gambusia as an agent in successfully applying a biological method of combating anopheline production in rice fields.

Sicault & Roule (1935), working in Morocco, found that if mosquitofish were present in numbers in excess of 20 per square meter they prevented all mosquito multiplication during the warm weather. However, in the spring and fall, when the temperature fell below 5° C., the fish sought the upper water and allowed the mosquitoes to breed freely in the shallow water.

De Buen (1940) found that, through their voracity, Gambusia, although not 100 per cent effective, kept the transmitters of malaria at a minimum in Spain.

Within recent years there has been considerable research on the habits of Gambusia and its ability to control mosquito production in different parts of the United States. In the Southwest the mosquitofish has been introduced into many places and has been found to be quite valuable in antimosquito work. Particularly in California this poeciliid has multiplied in large numbers and has become acclimatized in many parts of the state. In Utah (Rees 1934, 1945), where

difficulty was encountreed in holding the fish over winter, natural warm springs in the vicinity served as reservoirs for keeping the fish until the following summer. Recently, the mosquitofish survived in certain cold water ponds during mild winters.

In the vicinity of Chicago, Illinois, the Des Plaines Valley Mosquito Abatement District has been using Gambusia as an integral part of their campaign against "pest" mosquitoes since 1933. Each spring the various pools, marshes, and lakes of the region are checked for mosquitofish and if the population of the previous year has failed to survive the winter a new introduction is made. The acclimatization of the topminnow to northern Illinois and southern Michigan has been discussed previously. During late August 1938, the writer made examinations of 24 ponds southwest of Chicago which were suitable for fish during at least part of the summer. Early in the summer 14 of the ponds had been stocked with Gambusia and the remaining 10 had not. In those ponds in which no fish had been placed as a control measure, mosquito larvae and pupae, both anopheline and culicine, were taken in large numbers. In the 14 ponds where mosquitofish were present, only a few larvae were collected from 9 of the ponds and none could be found in the other 5.

The possible effectiveness of the mosquitofish in controlling the production of anopheline mosquitoes in southern Michigan was tested during 1943 in 4 small artificial ponds at the State Fish Hatchery at Drayton Plains. The ponds (Fig. 15) were built during the winter of 1936-37 and each was provided with a separate inlet and outlet. All of the ponds were similar in size, 100 by 50 feet, with maximum depths of about 4 feet. The water was shallow at the end where the inlet was located and became gradually deeper toward the outlet end. The bottoms of the ponds were mud to which some sand and gravel had previously been added. A heavy growth of Chara covered the pond floor. Some cattails and smartweeds grew in the shallower parts of each pond and some Najas was present in the deeper parts. Early in the summer the ponds were drained and all fish removed. Then the ponds were refilled and the aquatic vegetation was allowed to grow rampant for the rest of the summer so that natural ponds in the area might be simulated.

Each of the 4 ponds was inspected for mosquito larvae at approximately weekly intervals during the entire summer. One dip with a quart-size dipper, taken every 10 feet of shoreline assured an adequate sampling of the population of mosquito larvae in each pond. The contents of each dipperful was carefully scrutinized for aquatic stages in the life cycle of the mosquito and all specimens taken were grouped as larvae or pupae.

On July 23, 2 of the ponds (Ponds 7 and 8) were each stocked with 100 mosquitofish and the other 2 (Ponds 9 and 10) were used as controls. One of the control ponds (Pond 9) became polluted during the latter part of August and could no longer be used in the experiment. The cause of pollution was not found. The water was milky and had a foul odor and the animal life in the pond was at a minimum for the rest of the summer.

FIG. 15. Experimental minnow ponds at the Drayton Plains State Fish Hatchery. These ponds were used in testing the effectiveness of mosquitofish in controlling anopheline mosquitoes. (Courtesy of Michigan Institute for Fisheries Research.)

The experiment was repeated the following year using 6 ponds instead of 4. Accordingly, mosquitofish were stocked in 3 ponds (Ponds 6, 7, and 8) on July 14 and the other 3 ponds (Ponds 5, 9, and 10) served as controls. At the height of the anopheline breeding season (September 1), after the weekly collections had been made, 125 mosquitofish were seined out of Pond 7 and placed in one of the control ponds (Pond 9). Those fish appeared to be representative of the population in Pond 7 inasmuch as they ranged in size from newborn young to adult males and gravid females.

The mosquitofish brought about a considerable reduction in the abundance of the anopheline larvae and pupae in the hatchery ponds under observation (Tables 20 and 21; Fig. 16). The data indicate that Gambusia was 80.9 and 94.8 per cent effective in the control of anopheline mosquito production in 1943 and 1944 respectively. Furthermore, when the topminnows were introduced into a fish-tree pond in the middle of the mosquito breeding season the numbers of larvae and pupae decreased sharply in subsequent collections, whereas the numbers of larvae and pupae taken in the collections from the 2 remaining control ponds continued to increase for another 2 weeks. Although control was not as complete as that in ponds in which mosquitofish had been present during the entire summer, the production of Anopheles was greatly reduced.

In all probability the growth of aquatic vegetation in the hatchery ponds used in this experiment was not as rank as that in nearby natural waters of comparable size. However, the fact remains that Gambusia effected at least an 80 per cent control of anophelines breeding under experimental conditions which simulated those in nature. Also, the topminnows wintered over in the ponds and were present in

TABLE 20. Numbers of anopheline larvae and pupae collected from three ponds (the data from Pond 9 were not used) at the Drayton Plains State Fish Hatchery, 1943. Ponds 7 and 8 contained Gambusia and Pond 10 was used as a control.

Collection date	Pond 7		Pond 8		Pond 10	
	larvae	pupae	larvae	pupae	larvae	pupae
July 23	21	..	9	..	8	..
July 30	33	..	12	..	62	..
August 6	46	..	7	..	107	3
August 13	55	..	18	..	164	5
August 27	19	2	19	..	155	2
September 9	9	..	8	..	103	1
September 17	2	2	..	..	30	5
September 24	3	1	3	..	37	4
October 4	..	..	..	..	12	1
October 8	..	..	..	..	8	..
Total	188	5	76	..	686	21

sufficient numbers the following spring to supply an ample stock for further distribution of the species. Thus, the permanence of the control afforded by the use of the mosquitofish, together with the very low cost of introduction and maintenance, adds considerably to the practical value of this fish in antimosquito campaigns.

SUMMARY

A total of 30,093 specimens of *Gambusia a. affinis* were collected during 3 summers from 8 ponds in northeastern and central Illinois. All of those fish were measured, sexed, and classified as mature or immature individuals. The numbers of eyed embryos in the broods carried by 2,381 gravid females were counted.

Length, frequency, size distribution, and growth rate.—Female mosquitofish attain greater lengths than males because the females continue to grow until they die whereas males grow very little after the formation of the gonopodium is completed. Consequently, the largest specimen in any collection of Gambusia is usually a female.

Length frequencies and size distributions of mosquitofish in collections from Illinois depend to a great extent on the fertility of the pond and the time of

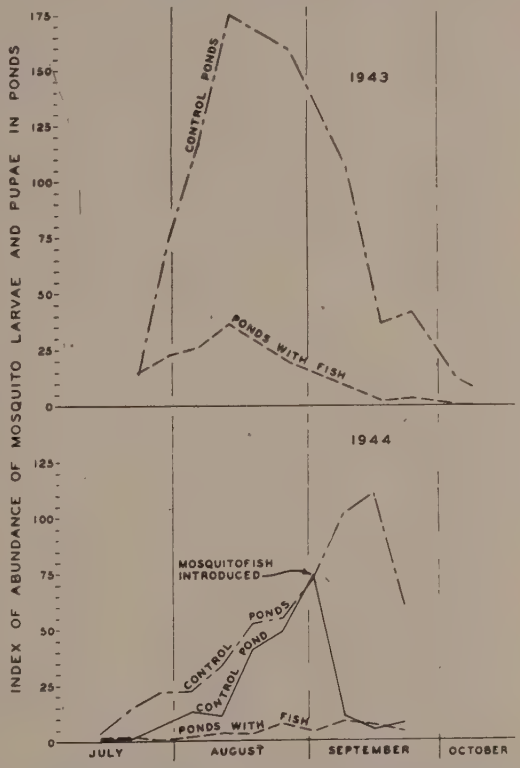

FIG. 16. Numbers of anopheline larvae and pupae collected from ponds at the Drayton Plains State Fish Hatchery, 1943 and 1944. The data represented in the graphs are given in Tables 20 and 21.

TABLE 21. Numbers of anopheline larvae and pupae collected from six ponds at the Drayton Plains State Fish Hatchery, 1944. Ponds 6, 7, and 8, contained Gambusia, and Ponds 5, 9, and 10, were used as control ponds. Gambusia were introduced into Pond 9 on September 1.

Collection date	Pond 6		Pond 7		Pond 8		Pond 9		Pond 5		Pond 10	
	larvae	pupae	larvae	pupae	larvae	pupae	larvae	pupae	larvae	pupae	larvae	pupae
July 14	..	..	3	..	1	..	1	..	..	..	6	1
July 21	..	..	4	..	..	..	1	..	6	..	23	..
July 28	..	..	..	..	1	..	6	1	5	..	38	1
August 4	3	..	1	..	..	..	13	..	6	1	38	..
August 11	4	..	..	..	3	..	11	..	10	1	54	2
August 18	1	..	5	..	..	..	40	1	27	..	63	4
August 25	3	..	5	..	7	..	48	1	30	..	80	..
September 1	..	..	5	1	2	..	70	4	52	1	92	..
September 8	5	..	4	2	6	..	11	..	64	..	131	8
September 15	4	..	8	..	2	..	5	..	69	..	151	2
September 22	4	..	3	..	1	..	8	..	40	4	68	8
September 29	3	..	2	..	2	..	8	..	33	1	65	7
October 6	2	..	3	..	..	..	2	..	20	..	44	4
Total	29	..	43	3	25	..	224	7	362	8	853	37

summer at which the collection is made. Samples taken during May contained only relatively large fish that had survived the preceding winter whereas young-of-the-year mosquitofish were invariably present in collections made later in the summer. Considerable differences in the growth rate of Gambusia, with consequent differences in size range, occurred in different ponds. Females, both parent and offspring, of the fast growing population in the fertile Argonne Woods Pond reached greater lengths and exhibited greater size ranges than did those in the less fertile Sanitary District Lake. In Parr's Pond, where the fertility was higher and the growth rate faster than in either of the Chicago ponds, too few parent females were taken to allow comparison but the female offspring attained a maximum length of 49 mm. during their first summer of life as compared with similar measurements of 44 mm. and 34 mm. respectively for female offspring from the Argonne Woods Pond and the Sanitary District Lake.

The largest mature male mosquitofish taken from the Sanitary District Lake was 36 mm. long as compared with maximum sizes of mature males of 32 mm. from the Argonne Woods Pond and 31 mm. from Parr's Pond. However, in all collections from those 3 ponds there were only 4 specimens more than 31 mm. long.

Accordingly, the maximum size of the female mosquitofish in Illinois depends largely on the fertility of the pond in which she lives whereas that of the male seldom exceeds 31 mm.

Size and age at first maturity.—Inasmuch as mosquitofish are promiscuous in their breeding habits the minimum size at which the young reach maturity is rather easily defined by the lengths of the smallest mature males and gravid females in adequate collections. The determination of the maximum size at which males become mature is obviated by the fact that growth practically ceases with the complete formation of the gonopodium. On the other hand, it is considerably more difficult to ascertain the maximum size at which females become gravid for the first time. Parent females, which were present in the ponds near Chicago at the beginning of the summer and had obviously survived the previous winter, became gravid for the first time early in their second summer of life. The maximum size for such females in collections from the Argonne Woods Pond was 45 mm. whereas those from the Sanitary District Lake were 31 mm. long. Those fish were 8-10 months old.

Some of the offspring of the parent fish became gravid during their first summer. The minimum sizes for such gravid offspring in the collections from the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond were 31 mm., 26 mm., and 24 mm. respectively. Those offspring were approximately 6 weeks old when they first became gravid.

Male mosquitofish reach maturity at smaller sizes and at slightly earlier ages than the females. The parent fish in the Argonne Woods Pond, the Sanitary District Lake, and Parr's Pond exhibited maximum lengths of 36, 32, and 31 mm. respectively whereas the offspring became mature at minimum lengths of 21, 21, and 19 mm. respectively.

Length of the reproductive period.—The female mosquitofish present in a wild population at the beginning of the summer in Illinois are virgin. Those fish become gravid for the first time at an age of 8-10 months and, after liberating a single series of broods, disappear from the population. Their reproductive period extends over 10-15 weeks depending on the number of broods liberated. Female offspring of the first and possibly the second brood liberated by the parent fish usually become gravid at an age of 4-6 weeks. These offspring, having been fertilized, give birth to a single series of broods and then die. Such offspring have a reproductive period 4-10 weeks long depending on the number of broods they bear.

The length of time males of Gambusia remain potent is not definitely known. It has been pointed out by other workers that the formation of sperm is a continuous process once maturity has been reached. Functionally, there is little need for male mosquitofish to retain their potency over long periods of time inasmuch as one fertilization of a female is adequate for the production of the single series of broods to which she gives birth.

Gestation period.—The gestation period for Gambusia ranged from 21 to 28 days in 14 such periods observed in the laboratory. The average for the 14 periods was 23.9 days.

Number of broods born per season.—The number of broods liberated by a female mosquitofish during a season in Illinois ponds depends on the age at which maturity is reached and, to a lesser extent, on the fertility of the pond in which she lives. Parent females, having reached maturity at an age of 8-10 months, give birth to a series of 3-4 broods whereas offspring that become mature when 4-6 weeks old, liberate only 1-2 broods. In Parr's Pond, where the population was newly established and the fertility of the pond was very high, the parent females gave birth to 4 and possibly 5 broods and the offspring liberated at least 3 broods of young.

Relationship between the length of the mother and the size of the brood.—In general, the average number of young per brood increases with the length of the mother fish. The physical limitation of the size of the mother fish is not the sole determining factor in the relationship between the gravid female and the number of young per brood. The rate of increase in the size of the brood is not constant with the rate of increase in the length of the mother fish but rather decreases as the length of the mother increases. Some metabolic factor, coincident with the approaching end of the reproductive season, apparently controls the rate of increase in the number of young per brood.

Decrease in the size of broods with increasing age.—The decrease in fecundity with increasing age holds for both parent fish and offspring. The average number of young per brood carried by females in Illinois

ponds showed that even though the actual length of the mother increased there was a marked decrease in the size of the broods in subsequent collections.

Senility.—Poeciliids, unlike many other fishes, have a definite period of sterile senility following reproduction. Senility among Gambusia is not a function of age alone. Females that began to bear young when only 4-6 weeks old were physiologically more senile at an age of 4 months than were females that did not begin to reproduce until they were 8-10 months old. Furthermore, the older fish reproduced over a longer period than did those which reached maturity at an early age.

Length of life under natural conditions.—In Illinois no mosquitofish was found that had survived 2 winters and entered the third summer of life (2 annuli on the scales). In general, mosquitofish die the same summer in which they reach maturity. The maximum life span is that of females which do not become mature until their second summer. Those fish liberate their series of broods and die at an age of about 15 months.

Sex ratio and differential death rate.—Male and female mosquitofish are born in approximately equal numbers. As the fish grow older there is a gradual shift in the sex ratio in favor of the females. The females are hardier and longer-lived than the males. Unequal sex ratios in collections of Gambusia are not to be attributed to a differential birth rate but rather to the relatively longer life and hardiness of the females.

Acclimatization to northern Illinois and to Michigan.—Gambusia native to southern Illinois have become acclimatized to natural waters in northern Illinois and have withstood the rigors of the climate in that region for more than 20 years. Some of the hardy stock from the Chicago area were introduced into outdoor ponds in southeastern Michigan in 1941 and have survived 6 winters there.

Mosquito control.—Experiments extending over 2 summers in Michigan showed that Gambusia was 80.9 and 94.8 per cent effective in the control of anopheline mosquito production in ponds where natural conditions were simulated. When introduced into a fish-free pond at the height of the mosquito breeding season, mosquitofish effected about an 80 per cent reduction in the anopheline reproduction within a week.

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